Universidad de Alcalá
COMISIÓN DE ESTUDIOS OFICIALES
DE POSGRADO Y DOCTORADO

ACTA DE EVALUACIÓN DE LA TESIS DOCTORAL

Año académico 2018/19

DOCTORANDO: **ARIAS ARIAS, ANGEL JESUS**
D.N.I./PASAPORTE: ******3858W**

PROGRAMA DE DOCTORADO: **D420-CIENCIAS DE LA SALUD**
DPTO. COORDINADOR DEL PROGRAMA: **BIOLOGIA DE SISTEMAS**
TITULACIÓN DE DOCTOR EN: **DOCTOR/A POR LA UNIVERSIDAD DE ALCALÁ**

En el día de hoy 29/04/19, reunido el tribunal de evaluación nombrado por la Comisión de Estudios Oficiales de Posgrado y Doctorado de la Universidad y constituido por los miembros que suscriben la presente Acta, el aspirante defendió su Tesis Doctoral, elaborada bajo la dirección de **ALFREDO JOSE LUCENDO VILLARIN // MARIA VICARIO P EREZ.**

Sobre el siguiente tema: *EPIDEMIOLOGÍA Y CARACTERIZACIÓN DE LA RESPUESTA INMUNITARIA INNATA EN LA ESOFAGITIS EOSINOFÍLICA. EFECTO DEL TRATAMIENTO DIETÉTICO CON DIETAS DE ELIMINACIÓN EMPÍRICAS.*

Finalizada la defensa y discusión de la tesis, el tribunal acordó otorgar la CALIFICACIÓN GLOBAL[1] de (**no apto, aprobado, notable** y **sobresaliente**): SOBRESALIENTE

Alcalá de Henares, 29 de ABRIL de 2019

EL PRESIDENTE

Fdo.: JOSE MARIA TENIAS BURILLO VAQUERO

EL SECRETARIO

Fdo.:ANGEL ASUNSOLO DEL BARCO

EL VOCAL

Fdo.: CECILIO SANTANDER

Con fecha 27 de mayo de 2019 la Comisión Delegada de la Comisión de Estudios Oficiales de Posgrado, a la vista de los votos emitidos de manera anónima por el tribunal que ha juzgado la tesis, resuelve:

☒ Conceder la Mención de "Cum Laude"
☐ No conceder la Mención de "Cum Laude"

La Secretaria de la Comisión Delegada

FIRMA DEL ALUMNO,

Fdo.: ARIAS ARIAS, ANGEL JESUS

[1] La calificación podrá ser "**no apto**" "**aprobado**" "**notable**" y "**sobresaliente**". El tribunal podrá otorgar la mención de "cum laude" si la calificación global es de sobresaliente y se emite en tal sentido el voto secreto positivo por unanimidad.

INCIDENCIAS / OBSERVACIONES:

Universidad de Alcalá
VICERRECTORADO DE INVESTIGACIÓN Y TRANSFERENCIA

En aplicación del art. 14.7 del RD. 99/2011 y el art. 14 del Reglamento de Elaboración, Autorización y Defensa de la Tesis Doctoral, la Comisión Delegada de la Comisión de Estudios Oficiales de Posgrado y Doctorado, en sesión pública de fecha 27 de mayo, procedió al escrutinio de los votos emitidos por los miembros del tribunal de la tesis defendida por ***ARIAS ARIAS, ANGEL JESUS***, el día 29 de abril de 2019, titulada, *EPIDEMIOLOGÍA Y CARACTERIZACIÓN DE LA RESPUESTA INMUNITARIA INNATA EN LA ESOFAGITIS EOSINOFÍLICA. EFECTO DEL TRATAMIENTO DIETÉTICO CON DIETAS DE ELIMINACIÓN EMPÍRICAS.* para determinar, si a la misma, se le concede la mención "cum laude", arrojando como resultado el voto favorable de todos los miembros del tribunal.

Por lo tanto, la Comisión de Estudios Oficiales de Posgrado **resuelve otorgar** a dicha tesis la

MENCIÓN "CUM LAUDE"

Alcalá de Henares, 31 de mayo de 2019
EL VICERRECTOR DE INVESTIGACIÓN Y TRANSFERENCIA
F. Javier de la Mata de la Mata

Copia por e-mail a:
Doctorando: ARIAS ARIAS, ANGEL JESUS
Secretario del Tribunal: ANGEL ASUNSOLO DEL BARCO
Directores de Tesis: ALFREDO JOSE LUCENDO VILLARIN // MARIA VICARIO PEREZ

Universidad de Alcalá

ESCUELA DE DOCTORADO
Servicio de Estudios Oficiales de Posgrado

DILIGENCIA DE DEPÓSITO DE TESIS.

Comprobado que el expediente académico de D./Dª ANGEL JESUS ARIAS ARIAS reúne los requisitos exigidos para la presentación de la Tesis, de acuerdo a la normativa vigente, y habiendo presentado la misma en formato: ☒ soporte electrónico ☐ impreso en papel, para el depósito de la misma, en el Servicio de Estudios Oficiales de Posgrado, con el nº de páginas: 238 se procede, con fecha de hoy a registrar el depósito de la tesis.

Alcalá de Henares a 5 de MARZO de 20 19

Aurora Juárez Abril

Fdo. El Funcionario

Universidad de Alcalá

Programa de Doctorado en
CIENCIAS DE LA SALUD

Epidemiología y caracterización de la respuesta inmunitaria innata en la Esofagitis Eosinofílica. Efecto del tratamiento dietético con dietas de eliminación empíricas.

Tesis Doctoral presentada por

ÁNGEL JESÚS ARIAS ARIAS

Directores:

Dr. Alfredo José Lucendo Villarín

Dra. María Vicario Pérez

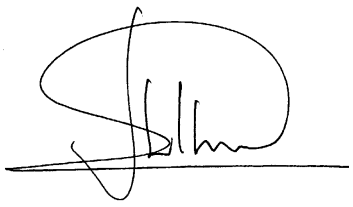
Alcalá de Henares, 2018/2019

El **Dr. D. ALFREDO JOSÉ LUCENDO VILLARÍN**, especialista en Aparato Digestivo, Jefe de Sección de Aparato Digestivo del Hospital General de Tomelloso (Ciudad Real)

CERTIFICA

Que la Tesis Doctoral titulada ***"Epidemiología y caracterización de la respuesta inmunitaria innata en la Esofagitis Eosinofílica. Efecto del tratamiento dietético con dietas de eliminación empíricas"*** que ha desarrollado **D. ÁNGEL JESÚS ARIAS ARIAS** bajo mi dirección reúne a mi juicio las características de originalidad, rigor metodológico, calidad científica y capacidad técnica e interpretativa por parte del autor, en condiciones tales que le hacen merecedora del Título de Doctor, siempre que así lo considere el Tribunal designado al efecto.

Y para que conste a los efectos oportunos, firma el presente certificado en Tomelloso, a 18 de Diciembre de 2018.



Fdo: Alfredo José Lucendo Villarín

La **Dra. Dª. MARÍA VICARIO PÉREZ**, investigadora asociada del Institut de Recerca Vall d'Hebron del Hospital Universitari Vall d`Hebron (Barcelona) Hospital Vall d`Hebron (Barcelona)

CERTIFICA

Que la Tesis Doctoral que presenta **D. ÁNGEL JESÚS ARIAS ARIAS** titulada ***"Epidemiología y caracterización de la respuesta inmunitaria innata en la Esofagitis Eosinofílica. Efecto del tratamiento dietético con dietas de eliminación empíricas"*** ha sido realizada íntegramente por el bajo mi dirección, y reúne a mi juicio las características de originalidad, rigor metodológico y capacidad técnica e interpretativa por parte del autor, en condiciones tales que le hacen merecedora del Título de Doctor, siempre que así lo considere el Tribunal designado al efecto.

Y para que conste a los efectos oportunos, firma el presente certificado en Barcelona, a 18 de Diciembre de 2018.

Fdo: María Vicario Pérez

Universidad de Alcalá

Dr. D. Pedro de la Villa Polo, Coordinador de la Comisión Académica del Programa de Doctorado en Ciencias de la Salud.

INFORMA que la Tesis Doctoral titulada **EPIDEMIOLOGÍA Y CARACTERIZACIÓN DE LA RESPUESTA INMUNITARIA INNATA EN LA ESOFAGITIS EOSINOFÍLICA. EFECTO DEL TRATAMIENTO DIETÉTICO CON DIETAS DE ELIMINACIÓN EMPÍRICAS** presentada por D. **ÁNGEL JESÚS ARIAS ARIAS**, bajo la dirección del Dr. D. Alfredo José Lucendo Villarín y de la Dra. Dña. María Vicario Pérez, ha sido realizada por compendio de artículos, reuniendo los requisitos exigidos a este tipo de tesis, así como los requisitos científicos de originalidad y rigor metodológicos para ser defendida ante un tribunal. Esta Comisión ha tenido también en cuenta la evaluación positiva anual del doctorando, habiendo obtenido las correspondientes competencias establecidas en el Programa.

Para que así conste y surta los efectos oportunos, se firma el presente informe en Alcalá de Henares a 05 de febrero de 2019.

UNIVERSIDAD DE ALCALÁ. PATRIMONIO DE LA HUMANIDAD

UNIVERSIDAD DE ALCALÁ · PD EN CIENCIAS DE LA SALUD · DEPARTAMENTO DE BIOLOGÍA DE SISTEMAS

Fdo.: Pedro de la Villa Polo

"No es el más fuerte de las especies el que sobrevive, tampoco es el más inteligente el que sobrevive. Es aquel que es más adaptable al cambio".

Charles Darwin

Para mis padres, Félix y María de los Ángeles,

Para mi hermano Félix,

Para Laura

AGRADECIMIENTOS

El desarrollo y realización de un trabajo de investigación o de una tesis doctoral es un camino prolongado y complejo que sin duda se puede llevar a cabo gracias a la colaboración de múltiples personas a las que siempre estaré agradecido:

En primer lugar quiero agradecer a mis directores de tesis los doctores María Vicaro y Alfredo Lucendo por su magnífica dirección y porque son unos científicos excelentes, a María por su disposición, por todos sus conocimientos, experiencia y por su magnífico talente. Una mención especial y mi eterno agradecimiento a Alfredo no solo por dirigir con maestría la presente tesis doctoral, si no por todo lo que he aprendido con él, por sus enseñanzas, por su genialidad y generosidad, sus consejos, ayuda y supervisión, por introducirme en el apasionante mundo la esofagitis eosinofílica y por que sin él este trabajo no hubiera sido imposible.

A todos los integrantes del laboratorio translacional de inmunología de la mucosa de la Dra Vicario en el Hospital Universitario del Vall de Hebrón, en especial a Marina Fortea y Ana María González por su importantísima contribución al presente trabajo en la realización de técnicas de biología molecular.

Al doctor David Bernardo y su grupo del Laboratorio de Investigación de Inmunología Intestinal de la Unidad de Enfermedad Inflamatoria Intestinal del Hospital Universitario de La Princesa por toda su colaboración y conocimientos imprescindibles en la finalización de este trabajo.

A todos los compañeros con las que he coincidido en algún momento en el Departamento de Investigación, Docencia y Formación (IDF) del Hospital General Mancha Centro, en especial a Oscar, por hacerme el trabajo más fácil y por convertirlo en un lugar al que, a pesar de las adversidades, da gusto ir todos los días. A Luis por su magnífica laboral al frente de la biblioteca y en la búsqueda de artículos y por todos esos momentos distendidos. Una mención especial a la Dra Olga Redondo por animarme en todo momento, por lo que he aprendido con ella y por todos los momentos que hemos compartido trabajando codo con codo.

Al doctor José María Tenias, por todo lo que he aprendido de él en todos los años que hemos coincidido en IDF, por su capacidad de trabajo, por ser la persona con más conocimientos de metodología y estadística que conozco y porque sin saberlo ni pretenderlo, hizo que quisiera seguir dedicándome con más fuerzas si cabe a la investigación, sobre todo a la investigación clínica.

Al servicio de Anatomía Patológica del Hospital Mancha Centro por toda su colaboración, y en especial a Teresa Mota, supervisora y técnico de Anatomía Patológica por la realización de sus magnificas preparaciones histológicas y tinciones, pero sobre todo por su amistad.

A todas las personas con las que he tenido la suerte de coincidir y colaborar a lo largo de toda mi trayectoria científica de las que he ido aprendiendo mucho, en especial a Belén Sáiz de Mier por todas sus enseñanzas de cómo trabajar en un laboratorio de investigación y por su amistad, a la Dra Trinidad Parra de la Unidad de Investigación de Guadalajara por darme mi primera oportunidad, por toda la confianza que depositó en mi y por enseñarme tanto, al igual que todo su equipo (Jaime, Selma y Miriam), a las doctoras Teresa Bellón y a Pilar Martínez del Hospital Universitario La Paz por su disponibilidad, amabilidad y aportaciones relevantes a los análisis de expresión génica.

A todos mis amigos, en especial a Quique y a Dani por todas las conversaciones científicas y de otra índole que hemos tenido durante la carrera y después de ella y por todas las que quedan por producirse; es decir, por su amistad, y también a Diana que, aunque nos hayamos conocido después, es como si la conociéramos de toda la vida.

A mis padres Félix y María de los Ángeles, por TODO, sin ellos nada tendría sentido y yo no sería como soy, por facilitarme todo, por todos sus sabios consejos y enseñanzas, por inculcarme la necesidad de esforzarse para conseguir los objetivos, por su cariño y por estar ahí siempre. A mi hermano Félix porque es un ejemplo a seguir, por todo su apoyo, por sus lecciones, consejos y recomendaciones siempre acertadas y en definitiva por el ser el mejor hermano que alguien puede tener.

Por último quería agradecer a Laura, aunque sea difícil expresar con palabras todo mi agradecimiento, gracias por ser como eres, por todo tu apoyo incondicional y por estar a mi lado en todos los momentos, por tu comprensión, por tu sonrisa y alegría eterna, por tu inmenso cariño, tu bondad y por todos los momentos que hemos vivido juntos y por todos los que nos quedan por vivir, muchas gracias de corazón y también a sus padres Enrique y Ascensión por su cariño, por preocuparse siempre por mi y por tratarme y cuidarme siempre tan bien.

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ABREVIATURAS

CCL11: Eotaxina 1 o citoquina (CC) ligando 11

CCL24: Eotaxina 2 o citoquina (CC) ligando 24

CCL26: Eotaxina 3 o citoquina (CC) ligando 26

CCR3: Receptor 3 de citoquinas CC

CGA: Campo de gran aumento

CRTH2: Molécula homóloga del receptor quimioatrayente expresada en células TH2

CXCL16: Citoquina (CXC) ligando 11

DAMP: Patrones moleculares asociados al daño

DE2A: Dieta de eliminación de 2 alimentos

DE4A: Dieta de eliminación de 4 alimentos

DE6A: Dieta de eliminación de 6 alimentos

ECO: Proteína catiónica del eosinófilo

EDN: Neurotoxina derivada del eosinófilo

EoE: Esofagitis eosinofílica

EPO: Peroxidasa eosinofílica

ERGE: Enfermedad por reflujo gastro-esofágico

EREFS: Score endoscópico de referencia para EoE

FCER1: Receptor de alta afinidad IgE

GZMA: Granzima A

GZMB: Granzima B

IBP: Inhibidor de la bomba protones

IBP-RE: Respondedores a IBPs

IgE: Inmunolglobulina E

IFN-α: Interferon α

IFN-β: Interferon β

IL-1α: Interleucina 1α

IL-1β: Interleucina 1β

IL-3: Interleucina 3

IL-4: Interleucina 4

IL-5: Interleucina 5

IL-6: Interleucina 6

IL-8: Interleucina 8

IL-9: Interleucina 9

IL-10: Interleucina 10

IL-13: Interleucina 13

IL-15: Interleucina 15

iNKT: Células T asesinas naturales invariantes

iNOS: Óxido nítrico sintetasa inducible

Kg: Kilogramos

KLRK1: *Killer cell lectin-like receptor subfamily K*

MAP-Kinasas: Proteínas quinasas activadas por mitógenos

MBP: Proteína mayor básica

MC: Mastocitos

mg: Miligramos

MICA: Secuencia relacionada con el polipéptido A del complejo mayor de histocompatibilidad

MICB: Secuencia relacionada con el polipéptido B del complejo mayor de histocompatibilidad

MT_T: Mastocitos con tripatasa

MT_{TC}: Mastocitos con triptasa/quimasa

MUC1: Mucina 1

MUC4: Mucina 4

MUC5B: Mucina 5B

MyD88: Respuesta primeria de diferenciación mieloide 88

NF-κβ: Factor nuclear kappa B

OR: Odds ratio

PCA: Análisis de componentes principales

PF: Propionato de fluticasona

PER-1: Perforina 1

RNAm: Ácido ribonucleico mensajero

SCF: *Stem cell factor*

SCFR: Receptor *Stem cell factor*

SNP: Single nuclotide polimorfism

STAT-6: Transductor de señal y activador de transcripción 6

T CD4+: Células T CD4 positivas

TGF-β: Factor de crecimiento transformante β

Th2: Linfocitos T *helper* 2

TLR: Receptor tipo Toll

TNF-α: Factor de necrosis tumoral α

TSLP: Linfopoyetina estromal tímica

µg: Microgramos

USA: United State of America

UTR: Región no traducida

INTRODUCCIÓN

INTRODUCCIÓN

1.- Concepto de Esofagitis Eosinofílica

La esofagitis eosinofílica (EoE) es una enfermedad esofágica crónica, local, mediada por mecanismo inmunológicos que se caracteriza clínicamente por síntomas relacionados con disfunción esofágica e histológicamente por una densa infiltración inflamatoria restringida al esófago, en la que predominan los granulocitos eosinófilos[1].

De manera específica, se diagnostica EoE ante un paciente con síntomas variados referidos a una alteración en la función del esófago (principalmente disfagia, impactación alimentaria, dolor abdominal, vómitos, pirosis y pérdida de peso), si en las biopsias obtenidas (generalmente mediante endoscopia digestiva) se documenta la infiltración del epitelio esofágico de al menos 15 eosinófilos por campo de gran aumento (CGA) en ausencia de infiltración eosinofílica en la mucosa del estómago y del duodeno. Para su diagnóstico debe, además, excluirse el consumo de fármacos, presencia de parásitos, causticación esofágica, neoplasias hematológicas y otras posibles casusas de eosinofília esofágica. Aunque hace años se precisaba además excluir reflujo gastroesofágico como causa de eosinofília esofágica, hoy conocemos que la EoE y la enfermedad por reflujo gastroesofágico (ERGE) pueden existir en un mismo paciente, de manera independiente o relacionada, si bien el segundo no determina una infiltración eosinofílica con la intensidad y las características que definen la EoE[2].

Los primeros casos de pacientes síntomas esofágicos y con infiltración eosinofílica en las biopsias de esófago[3,4] se comunicaron en la década de 1980, pero no fue hasta la década siguiente cuando la EoE se definió por primera vez como una entidad clínica diferenciada de la ERGE: En 1993, Stephen E Attwood y colaboradores[5] describieron 12 adultos jóvenes con disfagia e infiltración de eosinófilos restringida al esófago con características clínicas e histológicas propias y completamente diferentes a las presentes en la ERGE. Posteriormente, Alexander Straumann y colaboradores publicaron en 1994 una serie de 10 pacientes adultos con episodios de disfagia aguda recurrente que presentaban una densa infiltración eosinofílica esofágica[6]. Desde ese momento, la investigación, el conocimiento y la publicación (tanto científica como divulgativa) acerca de la EoE han ido aumentado exponencialmente hasta la actualidad, dando lugar a numerosos artículos (más de 2500 indexados en PubMed) que han abordado diversos aspectos clínicos, epidemiológicos y fisiopatológicos de la enfermedad.

El aumento en la frecuencia de la enfermedad ha requerido el desarrollo de hasta cinco guías clínicas para el diagnóstico y tratamiento de la EoE[1,7–10]. En la actualidad, la EoE es una de las patologías que más interés genera en las áreas clínicas de Gastroenterología y Alergología, aunque múltiples aspectos de la enfermedad permanecen desconocidos o poco estudiados, principalmente en lo referente a mecanismos celulares y moleculares, factores de riesgo, identificación de marcadores no invasivos y evolución a largo plazo.

2.- Epidemiología

Tras las primeras descripciones de la EoE la literatura médica ha proporcionado pruebas de un constante aumento en la frecuencia de la enfermedad en diversas poblaciones, hasta el punto de que la EoE representa actualmente el trastorno eosinofílico gastrointestinal más frecuente[11], así como la segunda causa más común de disfagia y esofagitis crónica después del ERGE, y la principal causa de síntomas esofágicos en niños y adultos jóvenes[12,13].

En los últimos años, numerosos estudios han estimado las cifras de incidencia y prevalencia de la enfermedad, principalmente en países occidentalizados de Norteamérica, Europa, así como Australia. Más recientemente también se han comunicado casos en Sudamérica, Asia y distintos países del norte de África, por lo que se puede hablar de que la EoE es ya una enfermedad de afectación global. Dichos estudios epidemiológicos se han realizado con distintos enfoques metodológicos, englobando estudios prospectivos y retrospectivos de registros de biopsias, series de endoscopias y estudios de base poblacional, siendo estos últimos los que presentan el diseño más adecuado para conocer los verdaderos valores de incidencia y prevalencia de la enfermedad.

El primer estudio epidemiológico retrospectivo de base poblacional realizado en Estados Unidos de América se publicó en 2004 por Noel y colaboradores[14], abarcaba un periodo de 4 años y estimó una incidencia de 10,7 nuevos casos por cada 100.000 habitantes/año y una prevalencia de 42,96 casos por 100.000 habitantes. Desde entonces, varios estudios de base poblacional posteriores desarrollados en el mismo país y en Europa han ido reportando cifras de prevalencia estables, de entre 30 – 56 pacientes por 100.000 habitantes, tanto en niños como en adultos[15–20], con unas tasas de incidencia ampliamente variables.

En el caso de España, contamos con pocos estudios de base poblacional para conocer la epidemiología de la EoE, si bien representan los más relevantes en el entorno europeo. El primero de ellos fue realizado por nuestro grupo en población adulta de Castilla-La Mancha para el periodo 2006-2011 y fue publicado en el año 2013[21]. La incidencia media anual fue de 6,4 nuevos casos por 100.000 habitantes y la prevalencia fue de 44,6 casos por 100.000 habitantes, lo que supondría un caso de EoE por 2250 adultos españoles, cifras que eran totalmente comprables a las reportadas en Suiza y en Norteamérica hasta esas fechas.

Una revisión sistemática con meta-análisis publicada en el año 2016 que resumió todos los estudios de base poblacional disponibles hasta esa fecha mostró una incidencia global de 3,7 nuevos casos por cada 100.000 habitantes, mientras que la prevalencia media estimada fue de 22,7 casos por 100.000 habitantes. Además, los autores mostraron que estas cifras aumentan considerablemente cuando se analizan sólo los datos obtenidos a partir del año 2008, con una incidencias muy variables de hasta 20 nuevos casos por 100.000 habitantes/año y una prevalencia de hasta 46 casos por 100.000 habitantes[22] **(Tabla 1).**

Este aumento de la frecuencia de la enfermedad, sobre todo en los últimos 5 - 10 años, ha sido documentado incluso en estudios desarrollados en una misma área geográfica a lo largo del tiempo. Por ejemplo, en el Condado de Olten (Suiza) se pasó de 23 a 42,8 casos/100.000 habitantes entre 2004 y 2009[15,16], o en el Condado de Olmsted (Minestota, USA) la prevalencia fue aumentando significativamente hasta los 54 casos por 100.000 habitantes[17].

Después de la publicación de la revisión sistemática antes referida se han publicados nuevos estudios de base poblacional que siguen proporcionando cifras similares de incidencia, cercanas a 20 nuevos casos por 100.000 habitantes/año[23] y cifras de prevalencias muy estables entorno a 50 casos por 100.000 habitantes[24,25]. En el último año también se han publicado estudios con tasas incidencia y, sobretodo, de prevalencia de EoE cada vez más elevadas. Por ejemplo, en un reciente estudio que investigaba la prevalencia de EoE en niños del estado de Utah (Estados Unidos) entre los años 2011 y 2016 ha documentado una prevalencia de 118 casos/100.000 habitantes y una incidencia de 24 nuevos casos pediátricos/100.000 habitante/año, siendo las cifras más altas reportadas en niños hasta la fecha[26]. En esta misma línea, un reciente trabajo realizado en Cáceres (España), documenta que la incidencia y prevalencia en adultos también siguen aumentando, situándose en cifras de 13,7 casos por 100.000 habitantes/año y de

81,7 casos por 100.000 habitantes, respectivamente, concluyendo que la frecuencia de la EoE se aproxima a la documentada para la enfermedad de Crohn en nuestro entorno[27] **(Tabla 1).**

No se conocen con exactitud las razones del rápido aumento en la epidemiología de la EoE, aunque algunos autores proponen que podría deberse al mayor conocimiento de la enfermedad por parte de los profesionales implicados en su diagnóstico y tratamiento (gastroenterólogos, alergólogos, patólogos y pediatras principalmente). Otros autores afirman que el uso creciente y rutinario de la endoscopia por en los sistemas de salud, así como la toma sistemática de biopsias podrían haber también contribuido a este aumento[19,28]. Sin embargo otros estudios muestran que este aumento en la frecuencia de diagnóstico de EoE supera al crecimiento en el número de endoscopias realizadas[27].

No obstante, este incremento es común para todas las enfermedades de tipo inmuno-alérgico y varias explicaciones tratan de justificar dicho aumento. La hipótesis de la higiene sostiene que las medidas higiénicas adoptadas en los países desarrollados han propiciado un ambiente más estéril por lo que los sistemas inmunes estarían expuestos a una menor variedad de antígenos y por tanto es menos probable que se desarrollen tolerancias. Por otra parte las hipótesis ambientales-geográficas mantienen que las áreas geográficas/climáticas y las exposiciones ambientales en las primeras etapas de la vida podrían influir en el desarrollo de este tipo de patologías[29–33]. En los últimos años el papel de la microbiota y los factores que la modifican también han adquirido una notable importancia a la hora de explicar el crecimiento en las cifras epidemiológicas[34].

La EoE puede presentarse en cualquier edad, aunque se ha observado que la mayor frecuencia de casos suele producirse entre los 30 y 50 años y principalmente afecta a personas de origen caucásico. También ha sido ampliamente reportado en la literatura una mayor afectación de hombres respecto a las mujeres, que viene a suponer que entre los primeros se produce el doble de casos, con un OR: 2,01 (IC95%: 1,63 – 2,48) según un estudio recientemente publicado[22].

Tabla 1: Principales estudios epidemiológicos de base poblacional realizados en EoE.

Estudio	País	Periodo	Tipo	Casos/población	Incidencia	Prevalencia
Noel R[14]	USA	2000 - 2003	Niños	103 / 239758	**12,8**	**42,96**
Cherian S[35]	Australia	1995 - 2004	Niños	285 / 3198653	**-**	**8,9**
Gill R[36]	USA	1995 - 2004	Niños	44 / 600000	**-**	**7,3**
Prasad G[17]	USA	1976 - 2005	Niños y Adultos	78 / 120000	**9,4**	**55**
Dalby K[37]	Dinamarca	2005 – 2007	Niños y Adultos	6 / 256164	**-**	**2,3**
Hruz P[15,16]	Suiza	1989 - 2009	Adultos	46 / 90000	**7,4**	**42,8**
Syed[19,20]	Canadá	2004 - 2008	Niños y Adultos	421 / 1250000	**10,7**	**33,7**
Arias A[21]	España	2005 - 2011	Adultos	40 / 89642	**6,4**	**44,62**
Van Rhijn B[38]	Holanda	1996 - 2010	Niños y Adultos	674 / 16615394	**1,3**	**4,05**
Prakash R[39]	USA	2010 – 2013	Niños	4680 / 14360300	**2,9**	**40**
			Adultos		**2,1**	**29**
Ally M[40]	USA	2008 – 2009	Niños	987 / 10180515	**5,2**	**10,5**
			Adultos		**4.7**	**9,5**
Dellon ES[18]	USA	2009 – 2011	Niños	6513 / 11569217	**16,8**	**50,5**
			Adultos		**19,6**	**58,9**
Dellon ES[41]	Dinamarca	1997 - 2011	Niños y Adultos	769 / 5572463	**2,6**	**13,8**

Tabla 1: Principales estudios epidemiológicos de base poblacional realizados en EoE. *(Continuación)*

Estudio	País	Periodo	Tipo	Casos/población	Incidencia	Prevalencia
Maradey-Romero C[24]	USA	2011 - 2014	Niños y Adultos	4840 / 9559570	-	**50,6**
Molina-Infante J[27]	España	2007 - 2016	Adultos	137 / 167620	**13,7**	**81,73**
Robson J[26]	USA	2011 - 2016	Niños	1060 / 895205	**24**	**118**
Hommeida S[23]	USA	2005 - 2015	Niños	73 / No especificado	**19,2**	-
Mansoor E[42]	USA	2010 - 2015	Niños y Adultos	7840 / 30301440	-	**25,9**
Giriens B[43]	Suiza	1993 - 2013	Niños y Adultos	179 / 743317	**6,3**	**24,1**
Kim S[25]	USA	2008 - 2013	Niños y Adultos	1561 / 3486069	-	**45**

3.- Historia Natural

En los últimos años han surgido diversos estudios que han abordado la historia natural de la EoE y que muestran que, en ausencia de tratamiento, puede conducir a una remodelación fibrosa del esófago, como consecuencia de la inflamación crónicamente mantenida, los fenómenos de transición epitelio-mesenquimal, fibrosis subepitelial y finalmente reducción del calibre esofágico, que clínicamente agrava los síntomas de disfunción del órgano[44]. Esto ha determinado que la EoE sea defina como una enfermedad progresiva, caracterizada por la evolución desde un fenotipo inflamatorio (habitualmente en la edad infantil) hacia un fenotipo fibro-estenótico (más típico de adultos), de forma similar a lo que ocurre en la enfermedad inflamatoria intestinal. La probabilidad de encontrar estenosis en el momento del diagnóstico además se doblaba por cada incremento de 10 años en la edad del paciente[45,46]. Si bien no todos los casos parecen evolucionar de acuerdo a esta secuencia, esta progresión en la que subyacen los procesos de remodelación fibrosa del esófago ha sido documentada de manera indirecta en la literatura. Así, se ha documentado que los pacientes con EoE sin tratamiento presentan un denso depósito de colágeno subepitelial que se correlaciona positivamente con el tiempo de progresión de los síntomas y su edad[47,48]. Este aspecto es crítico, ya que una reciente revisión sistemática ha estimado la demora promedio desde el inicio de los síntomas hasta el diagnóstico en 1,2–3,5 años en niños, y en 3-8 años en adultos[49]. Sin embargo, existen pruebas de que un tratamiento eficaz de la enfermedad puede limitar esta progresión[47-48,50-51].

Hasta la fecha no existe prueba alguna de que la EoE pueda estar asociada al desarrollo de algún tipo de degeneración maligna ni que sea potencialmente mortal[52]. Sin embargo, si está documentado que la EoE ejerce un impacto muy significativo sobre la calidad de vida de los pacientes, y en el caso de los pacientes pediátricos, también sobre la de sus familias[53–55]. Aunque son necesarios más estudios al respecto, los principales aspectos afectados son los relacionados con la función social y psicológica[56–58]; en el aspecto psicológico destacan la ansiedad por la enfermedad, por sufrir atragantamientos y por las consecuencias a largo plazo del curso crónico de la EoE. Entre los adultos el impacto social es más alto, en parte como consecuencia de las posibles restricciones en las actividades sociales que giran en torno a las comidas. El grado de afectación se correlaciona directamente con el tiempo de evolución de la enfermedad, así como con la gravedad (frecuencia e intensidad) de los síntomas[59–61].

4.- Diagnóstico

Ante un paciente con síntomas diversos referidos a una alteración continua o intermitente en la función del esófago, la marca histológica para el diagnostico de la EoE se basa en el recuento de eosinófilos por CGA en las biopsias obtenidas del epitelio esofágico. La densidad umbral en el número de eosinófilos por CGA necesarios para el diagnóstico de la EoE ha ido reduciéndose a lo largo del tiempo (empezó en 24 eosinófilos/CGA en la literatura más antigua, y posteriormente algunos artículos emplearon un umbral de 20) a partir del año 2011 éste ha sido establecido por consenso en ≥ 15 eosinófilos/cga[7]. Debido a la naturaleza parcheada de la enfermedad, para su correcto diagnóstico es necesario obtener, al menos 6 biopsias en dos localizaciones distintas del esófago (habitualmente proximal y distal) y si es posible, sobre las áreas de mayor alternación endoscópica, especialmente sobre los surcos longitudinales y los exudados[62,63]. La obtención de 6 biopsias posee una sensibilidad del 100% para el diagnóstico de la enfermedad y una especificidad del 96% para diferenciarla de la ERGE[64]. Al menos en la primera endoscopia, y siempre que existan síntomas asociados, se recomienda obtener también biopsias del estómago y duodeno para excluir otras patologías eosinofílicas concomitantes como la gastroenteritis eosinofílica. Además del recuento de eosinófilos, otros hallazgos histológicos pueden aportar una información adicional, útil en la discriminación diagnóstica de los casos complicados. Entre ellos se cuentan los microabscesos de eosinófilos en las capas más superficiales del epitelio, zonas de hiperplasia basal, espacios intracelulares dilatados, elongación de las papilas conjuntivas y fibrosis subepitelial y/o en la lámina propia. Se ha desarrollado un índice de actividad histológica para la EoE que pretende estandarizar la medición de estos parámetros y cuantificar la respuesta histológica al tratamiento, aunque todavía no han sido validados[58].

Si bien los eosinófilos son las células más representativas (y las más visibles con las tinciones de hematoxilina y eosina rutinariamente empleadas) el infiltrado inflamatorio esofágico en la EoE está integrado por más tipos celulares, incluyendo una mayor densidad de células T intraepiteliales[65], linfocitos B[66,67] y mastocitos[68,69], entre otros.

Junto con los hallazgos histológicos, existen diversas alternaciones endoscópicas en el esófago de los pacientes con EoE, que incluyen la presencia de surcos longitudinales, exudados blanquecinos, anillos esofágicos fijos, estenosis, pérdida del patrón vascular, edemas y estrechamiento en el calibre del esófago.

Adicionalmente algunos pacientes pueden mostrar mucosa frágil (que ha sido denominada como “en *paper crepe”,* con frecuentes desgarros esofágicos. Sin embargo, un porcentaje no desdeñable de pacientes (entre el 10 y el 30%, según diversos trabajos) pueden mostrar una mucosa esofágica de aspecto normal[70,71]. Con el objeto de homogeneizar y sistematizar la descripción de estos hallazgos endoscópicos se ha propuesto la clasificación y graduación EREFS **(Figura 1),** el acrónimo en inglés que indica edema, anillos, exudados, surcos longitudinales y estenosis[72]. Si bien esta clasificación mejora la descripción de los hallazgos, su descripción presenta una amplia variedad inter-explorador y no ha mostrado correlación suficiente para predecir la presencia o ausencia de inflamación eosinofílica, y ni siquiera predecir el diagnóstico no invasivo de la enfermedad, por lo que no puede sustituir a la evaluación histológica de las biopsias obtenidas a lo largo del esófago en el diagnóstico de un paciente con disfagia[73].

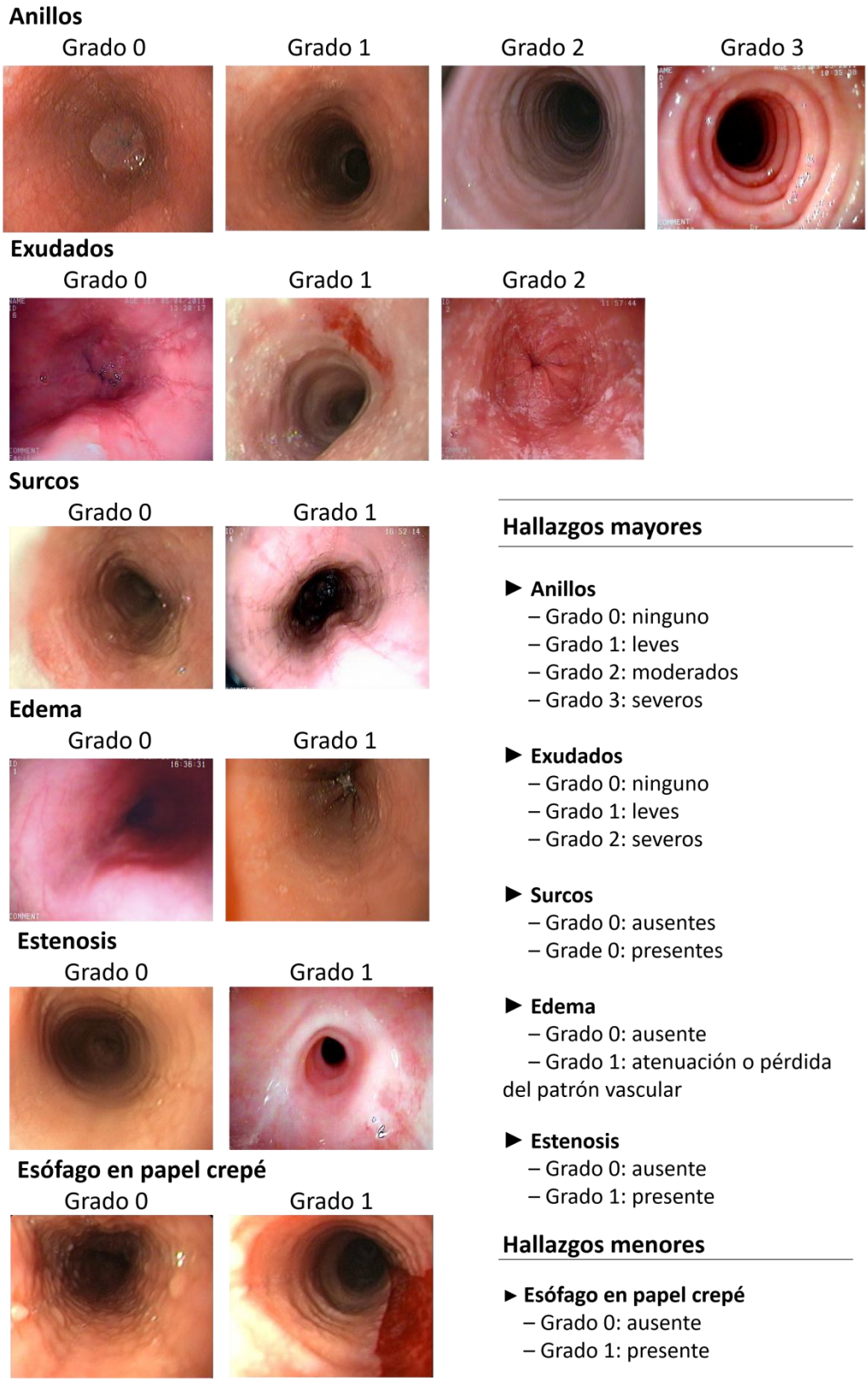

Figura 1. Clasificación y graduación de los hallazgos endoscópicos en la esofagitis eosinofílica.

Las principales manifestaciones clínicas de la EoE incluyen la disfagia, las impactaciones alimentarias, el dolor torácico y síntomas de remedan a la ERGE, en niños también son frecuentes los síntomas como vómitos, náuseas, pirosis, dolor torácico y abdominal y rechazo a la comida. Se han desarrollado destinas escalas de puntuación de síntomas tanto para niños como para adultos[74,75] que lamentable, no muestran correlación suficiente entre sus puntuaciones y la actividad histológica de la enfermedad, por lo que su uso en la monitorización de la EoE es muy limitado.

Hasta la fecha, no disponemos de marcadores no invasivos que puedan emplearse para el diagnóstico o en la monitorización de la actividad de la EoE a lo largo de su seguimiento. Todos los marcadores no invasivos que han sido propuestos y testados, incluyendo el número de eosinófilos en sangre, la concentración de IgE total, la proteína catiónica eosinofílica, la neurotoxina derivada de eosinófilos, la triptasa de mastocitos, algunas quimioquinas e incluso el oxido nítrico exhalado se han mostrado insuficientes[76–79].

En los últimos años, se han desarrollado herramientas mínimamente invasivas para el diagnóstico y la monitorización de la EoE, como el *String test* y la Citoesponga. El primero se basa en una derivación del Enterotest[80], y consiste en una capsula unida a un hilo de 90 cm de longitud, que permite impregnarse de las secreciones esofágicas y poder analizar en ellas las proteínas derivadas de los eosinófilos. La Citoesponja es una pequeña esponja de malla plegada dentro de una capsula de gelatina, que se expande una vez ingerida y tras su extracción por arrastre permite obtener células esofágicas para su análisis **(Figura 2)**. Todos estos dispositivos han mostrado resultados iniciales prometedores[81,82], cuya utilidad clínica debe ser confirmada en futuros estudios con mayor tamaño muestral.

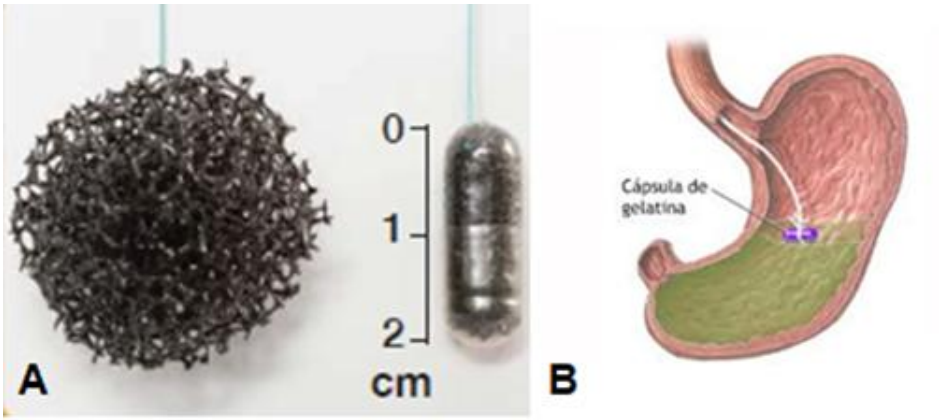

Figura 2: Dispositivos mínimamente invasivos utilizados en EoE**. A** Citoesponga. **B** String Test.

5.- Tratamiento

Los tratamientos empleados en los pacientes con EoE incluyen 3 grandes categorías: a) tratamientos dietéticos, b) tratamientos farmacológicos y c) dilatación esofágica. Los dos primeros poseen potencial efecto antiinflamatorio y deben emplearse como terapia de primera línea. La dilatación esofágica es un procedimiento mecánico que complementa a cada uno de los anteriores, pero carece de efecto antiinflamatorio por lo que no debe emplearse como única medida terapéutica[1].

5.1.- Tratamiento dietético

En 1995 Kevin Kelly y colaboradores, publicaron una serie de 10 niños con síntomas esofágicos graves atribuidos a ERGE y refractarios a las terapias habituales (en incluso en 6 casos con fracaso a fundusplicatura) asociada a un denso infiltrado eosinofílico en el epitelio esofágico. Tras la alimentación exclusiva con una fórmula elemental (compuesta exclusivamente por aminoácidos sin capacidad antigénica) durante al menos 8 semanas todos los niños resolvieron y mejoraron sus síntomas, mientras que la eosinofília esofágica también disminuyó significativamente; después de la reinstauración de dieta libre, la inflamación esofágica y los síntomas recurrieron en todos los casos[83]. Este trabajo constituyó el punto de partida para el tratamiento dietético de la EoE, capaz de prescindir completamente de fármacos y de reducir los costes para los sistemas sanitarios. Existen 3 modalidades de tratamiento dietético.

a) Alimentación exclusiva con dietas elementales

Las dietas elementales fueron empleadas ampliamente en el tratamiento de la EoE pediátrica, aunque más recientemente también han sido probadas en adultos. En todos los casos han mostrado una eficacia alta para resolver la EoE tanto en niños[70,84–92] como en adultos[93] **(Tabla 2)**. Una revisión sistemática situó a la dieta elemental como la opción de tratamiento dietético más eficaz en el tratamiento de la EoE, capaz de inducir la remisión histológica en el 90,8% (IC95%: 84,7% – 95,5%) de los pacientes[94].

Sin embargo, varias características limitan el uso de la dieta elemental exclusiva en la práctica clínica, incluyendo su alto coste, su sabor desagradable, los problemas con su adherencia debido a la necesidad de evitar cualquier otro alimento y las dificultades que impone en las relaciones sociales. En el primer estudio realizado en adultos, más de un tercio de los pacientes abandonaban la dieta elemental en las dos primeras semanas[93]. En el caso de los niños con EoE evitar los alimentos sólidos y su masticación retrasa el desarrollo de la musculatura facial y del habla[95] lo que representa una limitación adicional, por lo que no es recomendable para su uso crónico.

Por tanto, el único papel potencial propuesto para la dieta elemental está tras el fracaso otros tratamientos convencionales realizados de una manera óptima, en pacientes que desean permanecer en remisión mientras investigan el papel casual de alimentos inusuales o aeroalergenos en su enfermedad, pero este enfoque aún no ha sido evaluado[96].

Tabla 2: Principales estudios realizados con dietas elementales para el tratamiento de EoE.

Estudio	Periodo	Tipo	N	% Remisión
Kelly KJ[83]	1992 - 1994	Niños	10	**90%** (9/10)
De Agustín JC[84]	2002	Niños	2	**100%** (2/2)
Liacouras CA[70]	1994 - 2004	Niños	164	**98%** (160/164)
Ferreira CT[85]	2008	Niños	1	**100%** (1/1)
Rizo-Pascual JM[86]	2001 -2009	Niños	3	**100 %** (3/3)
Basilious A[87]	2005	Niños	2	**100 %** (2/2)
Abu-Sultaneh SM[88]	2003 – 2008	Niños	1	**0 %** (0/1)
Kagalwalla AF[89]	2006 - 2011	Niños	12	**83%** (10/12)
Peterson KA[93]	2009 - 2011	Adultos	18	**94%** (17/18)
Henderson CJ[90]	1999 - 2011	Niños	49	**96%** (47/49)
Spergel JM[91]	2000 -2011	Niños	151	**95%** (144/151)
Al-Hussaini A[92]	2009 - 2012	Niños	3	**100%** (3/3)
Hiremath G[97]	-	Niños	13	**62%** (8/13)
Warners MJ[98,99]	2014 - 2015	Adultos	17	**94%** (16/17)

b) Dietas dirigidas por los resultados de pruebas de alergia

Una vez que se confirmó que la EoE está causada por alimentos y que las dietas elementales (sin capacidad antigénica) eran capaces de inducir su remisión clínico-patológica en la gran mayoría de los pacientes y debido a las limitaciones de la dieta elemental, surgió la alternativa de excluir alimentos que resultaban positivos en diferentes pruebas de alergia. Esta estrategia consiste en tratar de identificar los alimentos desencadenantes de la EoE mediante test epicutáneos con parches (*pacht test*), test de *prick* cutáneos o frente a los cuales se detecta IgE específica sérica, comprobando la remisión de la enfermedad tras retirar de la dieta del paciente los alimentos con resultados positivos.

La primera experiencia con esta estrategia dietética fue publicada en 2002 por Jonathan M Spergel y colaboradores[100], quienes excluyeron de las dietas de 24 niños aquellos alimentos que resultaron positivos tras una combinación de pruebas de *prick* cutáneas y parches epicutáneos. Los pacientes presentaron de media 5 alimentos a excluir por resultados positivos, pero esta estrategia del estudio se realizó sólo en aquellos con pocos alimentos; la eficacia de la intervención en ellos fue del 49%. Los niños con 5 o más alimentos positivos fueron tratados con dieta elemental, y la eficacia global resultado de combinar ambas terapias fue del 77%. Tras esta primera experiencia, otros muchos estudios han utilizando las dietas de eliminación basadas en pruebas de alergia en niños[70,86,87,89–92,101] y en adultos[102,103], logrando un porcentaje de remisión global subóptimo. Un meta-análisis de los estudios publicados hasta 2014 **(Tabla 3)** mostró una eficacia global del 45,5% (IC95%: 35,4% - 55,7%), siendo menor ésta eficacia en adultos que en niños, y con una amplia heterogeneidad[94].

Tabla 3: Principales estudios realizados con dietas dirigidas por pruebas de alergia para el tratamiento de EoE.

Estudio	Periodo	Tipo	N	% Remisión
Siafakas CG[104]	2000	Niños	1	**100%** (1)
Liacouras CA[70]	1994 - 2004	Niños	75	**24%** (18/75)
Arirola-Pereda G[105]	2006	Niños	2	**100%** (2/2)
Quagletta L[101]	2005 - 2006	Niños	7	**0%** (0/7)
Rizo-Pascual JM[86]	2001 -2009	Niños	11	**45%** (5/11)
Basilious A[87]	2005	Niños	2	**50%** (1/2)
Kagalwalla AF[89]	2006 - 2011	Niños	82	**63%** (52/82)
Henderson KA[90]	1999 - 2011	Niños	23	**65%** (15/23)
Molina-Infante J[102]	-	Adultos	15	**27%** (4/15)
Spergel JM[91]	2000 -2011	Niños	319	**53%** (169/319)
Al-Hussaini A[92]	2009 - 2012	Niños	10	**40%** (4/10)
Kewalramani A[106]	-	Niños	13	**46%** (6/13)
Kalach N[107]	-	Niños	49	**53%** (26/49)
Wolf WA[103]		Adultos	17	**35%** (6/17)

La baja eficacia, sobre todo en adultos, unido a los pobres niveles predictivos que han mostrado las pruebas de alergia para identificar los alimentos causantes de la EoE[108], hacen desaconsejar su uso general en el tratamiento de la EoE.

c) Dietas empíricas

Debido a que las dietas dirigidas por pruebas de alergia han mostrado gran heterogeneidad en sus resultados y bajas tasas de remisión de la enfermedad y las desventajas ya mencionadas de las dietas elementales, se hacía necesario un enfoque alternativo en el tratamiento dietético de la EoE.

En 2006 el grupo liderado por Amir Kagalwalla, un pediatra de Chicago, publicó los resultados de un estudio en el que excluyo de la dieta de 35 niños con EoE los 6 alimentos más frecuentemente relacionados con alergia alimentaria (leche de vaca, trigo, huevo, soja, frutos secos y pescados y mariscos), independientemente de los resultados de las pruebas de alergia[109]. Esta dieta empírica eliminación de 6 alimentos (DE6A) consiguió la remisión histológica de la enfermedad en el 74% de los niños. Posteriores estudios han reproducido homogéneamente estos resultados en series pediátricas[90] y también en adultos[103,108,110], obteniéndose la remisión de la EoE en tres cuartas partes de los pacientes. La revisión sistemática sobre el tratamiento dietético de la EoE publicada en 2014 mostró una tasa de remisión global de la EoE del 72% (IC95%: 66% - 78%) y una amplia homogeneidad entre los estudios[94].

Además de inducir la remisión, la DE6A ha permitido identificar específicamente los alimentos causantes de la enfermedad en cada paciente individual mediante su reintroducción secuencial y la realización de endoscopias con biopsias repetidas, permitiendo además conocer las causas alimentarias más habituales de la EoE. Gracias a esta estrategia, sendos estudios han identificado la leche de vaca y el gluten como los principales causantes de la enfermedad seguidos del huevo y las legumbres y/o la soja[108,110].

La principal limitación de la dieta de 6 alimentos deriva de las numerosas endoscopias que precisa el paciente, el alto nivel inicial de restricciones alimentarias (la mayoría innecesarias), el periodo largo periodo para completar el proceso lo que hizo preciso desarrollar estrategias de dietas empíricas más simples. En este contexto, surgió la dieta de eliminación empírica de 4 alimentos (DE4A), donde restringe de la dieta del paciente los 4 grupos alimentos que más frecuentemente causan la EoE (leche de vaca, gluten, huevo y legumbres/soja). La primera experiencia publicada con la DE4A fue publicada en 2014 por Javier Molina-Infante y colaboradores. Un grupo de 52 pacientes adultos españoles con EoE mostraron una tasa de remisión del 54%[111]. Muy recientemente Kagalwalla y colaboradores han replicado esta estrategia en un

estudio sobre 78 niños con EoE de Estados Unidos, mostrando una tasa de remisión global del 64%[96].

Con el fin de simplificar el proceso y reducir aún más la necesidad de endoscopias y dado que la mayoría de los casos de EoE tienen a la leche, al gluten o a ambos como causa de su enfermedad, recientemente se ha testado una dieta de eliminación de 2 alimentos (DE2A) con una posterior estrategia secuencial de DE4A y DE6A en los pacientes con EoE que no alcanzaban la remisión clinicopatológica. Tras eliminar leche y gluten en 130 pacientes españoles con EoE de todas las edades la DE2A consiguió la remisión de la enfermedad en el 43% de los casos. En los no respondedores, el uso secuencial de una DE4A y de una DE6A consiguió una remisión global de la EoE en el 60 y 79% de los pacientes, respectivamente. Esta estrategia progresiva redujo la duración del proceso diagnóstico y el número de endoscopias necesarias en un 20%, respecto a la alternativa de comenzar por una DE6A[112]. Las ventajas de este nuevo esquema secuencial los sitúan como la elección para el tratamiento de primera línea en pacientes con EoE **(Tabla 4)**.

Tabla 4: Principales estudios realizados con dietas empíricas de eliminación para la EoE

Estudio	Periodo	Tipo	Dieta eliminación	N	% Remisión
Quagletta L[101]	2005 - 2006	Niños	Dieta sin gluten	3	**100%** (3/3)
Ooi CY[113]	1999 - 2007	Niños	Dieta sin gluten	2	**0%** (0/2)
Verzegnassi F[114]	2006	Adultos	Dieta sin gluten	2	**50%** (1/2)
Leslie C[115]	2000 - 2007	Niños	Dieta sin gluten	4	**0%** (0/4)
Al-Hussaini A[92]	2009 - 2012	Niños	Dieta sin gluten	1	**100%** (1/1)
Costable J[116]	2012	Adultos	Dieta sin gluten	1	**100%** (1/1)
Johnson JB[117]	2010	Adultos	Dieta sin gluten	2	**100%** (2/2)
Kagalwalla AF[89]	2006 - 2011	Niños	Dieta sin leche	17	**65%** (11/17)
Alonso-Llamazares A[118]	2010	Adultos	Dieta sin leche	1	**100%** (1/1)
Maggadottir SM[119]	2012	Niños	Dieta sin leche	1	**100%** (1/1)
Abu-Sultaneh SM[88]	2003 – 2008	Niños	Dieta sin soja	1	**100%** (1/1)
Kagalwalla AF[109]	2003 - 2005	Niños	DE6A	35	**74%** (26/35)
Kagalwalla AF[89]	2006	Niños	DE6A	1	**100%** (1/1)
Gonsalves N[110]	2006 - 2010	Adultos	DE6A	50	**74%** (37/50)
Henderson CJ[90]	1999 - 2011	Niños	SFED	26	**81%** (21/26)
Lucendo AJ[108]	2008 - 2010	Adultos	DE6A	67	**73%** (49/67)
Miur RJ[120]	-	Niños	DE6A	13	**54%** (7/13)
Wolf WA[103]		Adultos	DE6A	5	**40%** (2/5)
Molina-Infante J[111]	2012 - 2014	Adultos	DE4A	52	**54%** (28/52)
			DE6A + DE4A	47	**72%** (34/47)
Molina-Infante J[112]	2014 - 2016	Niños y Adultos	DE2A	130	**43%** (56/130)
			DE6A + DE4A	110	**60%** (66/110)
			DE2A+DE4A+DE6A	93	**79%** (74/93)
Kagalwalla AF[121]	2011 - 2016	Niños	DE4A	78	**64%** (50/78)

5.2.- Tratamiento farmacológico

A lo largo de la corta historia de la EoE se han ensayo diversos tratamientos farmacológicos para alcanzar la remisión clinicopatológica de la enfermedad y su posterior mantenimiento, que se pueden resumir en los siguientes:

a) Inhibidores de la Bomba de Protones (IBP)

Los IBP constituyen la primera línea de tratamiento de la ERGE[122], y las primeras guías clínicas sobre EoE exigían una prueba terapéutica con este fármaco a dosis dobles para excluir eosinofílica esofágica determinada por reflujo gastroesofágico[7–10], diagnosticando EoE en caso de persistencia del infiltrado inflamatorio. La definición de la eosinofília esofágica respondedora a IBP y su posterior independencia de la exposición ácida del esófago abrió el camino al actual concepto de la EoE[1] como una enfermedad que puede coexistir con la ERGE, pero de cual se distingue incluso por su firma genética[123], y a los IBP como una alternativa de primera línea para el tratamiento de estos pacientes.

Repetidos estudios han documentado la capacidad de los IBP para inducir y mantener la remisión clínica e histológica de la EoE en pacientes de todas las edades[124,125]. Desde la primera serie prospectiva publicada en 2011 en la que el 75% de los pacientes alcanzaban una remisión clínica e histopatológica con este fármaco[102], varios estudios prospectivos y retrospectivos[126–129] **(Tabla 5)** han documentado que el 50% de los pacientes tratados con IBP alcanzan la remisión histopatológica y en un 60% remiten los síntomas, de acuerdo con una revisión sistemática[130]. Las dosis recomendadas de omeprazol en adultos son de 20 – 40 mg dos veces al día o equivalentes, y de 1 – 2 mg/kg (o equivalentes para otros IBP) en los niños.

El tratamiento con IBPs es también efectivo para mantener la remisión clínicopatológica en el largo plazo. En niños, un reciente estudio prospectivo ha demostrado que la mayoría (78%) de los tratados con IBP mantenía su enfermedad en remisión tras un año empleando dosis bajas[131]. En adultos, un estudio multicéntrico

replicaba estos resultados, con un 73% de casos con su enfermedad controlada tras 1 año de tratamiento a doses estándar de IBP[132]. Los pacientes que perdieron respuesta fueron capaces de recuperarla al regresar a dosis dobles del fármaco.

Los IBP poseen una actividad anti-inflamatoria que se ha demostrado recientemente en diversos estudios[133–135]. Debido a la eficacia aceptable de los IBP, su reducido coste y su seguridad, constituyen un tratamiento de primera línea para la EoE[1].

Tabla 5: Principales estudios realizados con IBPs para el tratamiento de pacientes con EoE.

Estudio	N	Tipo	Tipo IBP	Dosis	Tiempo	% Remisión Histológica	% Remisión Clínica	Periodo
Nurko S[136]	8	Niños	No especificado	-	> 4 semanas	-	**75%** (6/8)	1990 -2002
Cury EK[137]	1	Niños	Lansoprazol	-	-	**0%** (0/1)	**0%** (0/1)	-
Potter JW[138]	12	Adultos	No especificado	-	-	-	**25%** (3/12)	1999- 2002
Ngo P[139]	3	Niños	Omeprazol	10*	8 semanas	**100%** (1/1)	-	-
		Niños		20	8 semanas	**100%** (1/1)	**100%** (1/1)	
		Adultos		20*	Varias semanas	**100%** (1/1)	-	
Nantes O[140]	3	Adultos	No especificado	-	-	**0%** (0/3)	**100%** (3/3)	2002 - 2008
Sayej WN[124]	36	Niños	No especificado	1-2 mg/Kg/d	12 semanas	**38,9%** (14/36)	**77,8%** (28/36)	2003 -2008
Gortani G[141]	1	Niños	Lansoprazol	15	6 semanas	**100%** (1/1)	**100%** (1/1)	-
Dranove JE[125]	43	Niños	No especificado	-	-	**39,5%** (17/43)	**86%** (37/43)	1999 - 2006
Garrean CP[142]	64	Adultos	No especificado	-	-	**25%** (16/64)	-	-
Peterson KA[126]	15	Adultos	Esomeprazol	40	8 semanas	**50%** (6 /12)	**25%** (3/12)	2005 - 2006

Tabla 5: Principales estudios realizados con IBPs para el tratamiento de pacientes con EoE. *(Continuación)*

Estudio	N	Tipo	Tipo IBP	Dosis	Tiempo	% Remisión Histológica	% Remisión Clínica	Periodo
Jung YM[143]	2	Adultos	Omeprazol	20	8 semanas	-	**50%** (1/2)	2006 - 2008
Molina-Infante J[144]	35	Adultos	Rabeprazol	20*	8 semanas	**74,3%** (26/35)	**74,3%** (26/35)	2008
Abe Y[145]	6	Adultos	No especificado	-	-	**50%** (3/6)	**83,3%** (5/6)	2006 - 2009
Fujiwara Y[146]	5	Adultos	Rabeprazol	10	8 semanas	**60%** (3/5)	**60%** (3/5)	2010 - 2011
Dohil R[147]	3	Niños	Lansoprazol	15*	12 semanas	**100%** (1/1)	**100%** (1/1)	-
			Lansoprazol	20*	12 semanas	**100%** (1/1)	**0%** (0/1)	
			Lansoprazol	30	-	**100%** (1/1)	**0%** (0/1)	
Levy AN[148]	1	Adultos	Omeprazol	40*	6 semanas	**0%** (0/1)	**0%** (0/1)	-
Francis DL[149]	18	Adultos	Esomeprazol	40*	6 semanas	**61,1%** (11/18)	**27,8%** (5/18)	2009 - 2010
Cohen-Sabban J[150]	23	Niños	No especificado	-	-	-	**30,4%** (7/23)	2007 - 2008
Vázquez-Elizondo G[127]	60	Adultos	Omeprazol	20*	8 semanas	**56,7%** (34/60)	**71,7%** (43/60)	2008 - 2012
Tomomatsu Y[151]	6	Adultos	No especificado	-	-	-	**33,3%** (2/6)	2010 - 2011
Schroeder S[152]	35	Niños	No especificado	1-2 mg/Kg/d	8 semanas	**22,9%** (8/35)	**22,9%** (8/35)	2000 - 2011
Rea F[153]	25	Niños	No especificado	-	8 semanas	**60%** (15/25)	-	2005 - 2011

Tabla 5: Principales estudios realizados con IBPs para el tratamiento de pacientes con EoE. *(Continuación)*

Estudio	N	Tipo	Tipo IBP	Dosis	Tiempo	% Remisión Histológica	% Remisión Clínica	Periodo
Moawad FJ[128]	21	Adultos	Esomeprazol	40	8 semanas	**33,3%** (7/21)	-	2008 - 2010
Lee JH[154]	6	Adultos	No especificado	-	4 - 8 semanas	**83,3%** (5/6)	**33,3%** (2/6)	2006 - 2011
Martinek J[155]	26	Adultos	No especificado	20*	Largo plazo	-	**96,2%** (25/26)	-
Dellon ES[129]	66	Adultos	No especificado	20 – 40*	8 semanas	**36,4%** (24/66)	-	2009 - 2011
Yilmaz O[156]	2	Niños	Esomeprazol	20	48 semanas	**0%** (0/1)	**100%** (1/1)	-
			No especificado	-	12 semanas	**0%** (0/1)	**100%** (1/1)	
Mangla S[157]	17	Adultos	No especificado	Dosis Altas*	8 semanas	**64,7%** (11/17)	-	2013
Lipka S[158]	1	Adultos	Rabeprazol	20*	4 semanas	**100%** (1/1)	**100%** (1/1)	-
Molina-Infante J[159]	53	Adultos	Omeprazol	40*	8 semanas	**43,4%** (23/53)	**43,4%** (23/53)	2010 - 2013
Dhaliwal J[160]	6	Niños	No especificado	1 mg/Kg/d*	-	**83,3%** (5/6%)	**100%** (6/6)	1999 - 2006
Van Rhijn BD[161]	16	Adultos	Esomeprazol	40*	8 semanas	**50%** (8/16)	-	-
Yamada Y[162]	3	Niños	No especificado	-	-	**100%** (3/3)	-	2005 - 2013

b) Glucocorticoides sistémicos

El tratamiento con corticoides sistémicos (prednisona y prednisolona) fue la primera opción farmacológica empleada en el manejo de la EoE para inducir la remisión de la inflamación del órgano. Los corticoides sistémicos son efectivos tanto en niños como en adultos y logran la remisión de la enfermedad en la mayoría de los pacientes. Sin embargo, tras suspender el tratamiento se produce la recurrencia de los síntomas y de la inflamación, haciendo precisos ciclos repetidos de esteroides[163–165].

En 1998 se publicó la eficacia de los corticoides inhalados de aplicación tópica deglutida en cuatro niños con EoE[166]. Posteriormente un ensayo clínico aleatorizado comparó la eficacia de prednisona oral (1 mg/kg dos veces al día) frente a propionato de fluticasona deglutido (2 inhalaciones 4 veces al día, 110 µg por inhalación en niños de menos de 10 años y 220 µg por inhalación para niños mayores de 11 años) durante 12 semanas en una serie de niños con EoE. Ambos tratamientos resultaron igual de efectivos para alcanzar la remisión clínico-histológica[163], sin ventajas de la primera opción en cuanto al tiempo hasta la recidiva, pero con notables efectos adversos sobre los corticoides tópicos, que aparecieron en casi la mitad de los niños. Por este motivo, los corticoides sistémicos no están recomendados para el tratamiento de la EoE y han quedado exclusivamente restringidos a situaciones de emergencia y/o en pacientes con síntomas graves que precisan control rápido.

c) Tratamiento con glucocorticoides tópicos

Tras demostrarse la eficacia de los corticoides tópicos en la EoE, estos llegaron a convertirse en el tratamiento de referencia (antes de contar con las dietas empíricas y los IBP), y en la actualidad continúan siendo uno de los tratamientos de primera línea para pacientes de todas las edades[1]. Los fármacos más utilizados son propionato de fluticasona y budesonida en distintas formulaciones, capaces de lograr la remisión de la enfermedad si se emplean a las dosis apropiadas y, lo que es más importante, con un vehículo capaz de conducirlos hasta el esófago[167]. En todo caso, su

eficacia depende de la administración continuada y por norma la enfermedad recurre tras su suspensión.

Numerosos estudios observacionales prospectivos y retrospectivos y hasta 12 ensayos clínicos aleatorizados demuestran la eficacia de los esteroides tópicos para conseguir la remisión histológica y, en menor medida, clínica de la EoE. El primer ensayo se publicó en 2006 y comparó propionato de fluticasona con placebo en niños con EoE, observándose de remisión histológica (definida por <1 eosinófilo/CGA) en el 47%, y clínica en el 67% de los tratados[168]. Ensayos posteriores han mostrado tasas más altas de remisión tanto con propionato de fluticasona como con budesonida viscosa, en niños y adultos **(Tabla 6).** Además se han publicado varias revisiones sistemáticas con meta-análisis que han resumido los resultados de estos ensayos clínicos concluyendo todas ellas que los corticoides tópicos son eficaces para alcanzar la remisión de la EoE, haciendo también hincapié en la variabilidad en los criterios de inclusión/exclusión, dosificación, presentación, tiempo de tratamiento y remisión histológica entre los distintos ensayos, que complica las comparaciones directas entre ellos[169–172].

Menos estudios han evaluado la eficacia de los corticoides para mantener la remisión clínica a lo largo del tiempo[50,173,174], que suele mantenerse mientas persista la medicación. Hasta la fecha los corticoides tópicos a largo plazo se han mostrado seguros, y no se han comunicado hasta la fecha eventos adversos relevantes[175], siendo el más común la candidiasis esofágica que puede ocurrir aproximadamente en el 10% de los pacientes, si bien muchas son asintomáticas y se diagnostican durante las endoscopias de seguimiento realizadas por protocolo.

Tabla 6: Principales ECA con esteroides tópicos para tratamiento de pacientes con EoE. PF: Propionato de fluticasona; cga: campo gran aumento

Estudio	Tipo Pb	Grupo Experimental				Grupo Control				Remisión Histológica		Remisión Clínica	
		N	Fármaco	Dosis	Duración	N	Fármaco	Dosis	Duración	Experimental	Control	Experimental	Control
Konikoff [168]	Niños	21	PF	880 µg	12 semanas	15	Placebo		12 semanas	*< 1 eosinófilo/cga*		*Resolución de Vómitos*	
										47% (10 / 21)	**6,7%** (1/15)	**67%** (14/21)	**27%** (4/15)
Schaefer [163]	Niños	40	PF	880 µg	4 semanas	40	Prednisona oral	1 mg/kg/día	4 semanas	*< 1 eosinófilo/cga*		No hay diferencias	
										45% (18/40)	**65%**(26/40)		
Dohil R [176]	Niños	15	Budesonida viscosa	1 mg ó 2 mg	12 semanas	9	Placebo		12 semanas	*< 6 eosinófilos/cga*		1,2 ± 1,87	1,85 ± 2,67
										86% (13/15)	**0%** (0/9)		
Straumann A[177]	Adultos	18	Budesonida viscosa	4 mg	2 semanas	18	Placebo		2 semanas	*< 5 eosinófilos/cga*		2,22 ± 2,07	4,72 ± 1,96
										72% (13/18)	**11%** (2/18)		
Peterson KA[126]	Adultos	15	PF	880 µg	8 semanas	15	Esomeprazol	40 mg	8 semanas	*< 5 eosinófilos/cga*		1,7	2,3
										13% (2/15)	**27%** (4/15)		
Dellon ES [167]	Adultos	11	Budesonida viscosa	2 mg	8 semanas	11	Budesonida inhalada	2 mg	8 semanas	*< 1 eosinófilo/cga*		*Cuestionario Disfagia Mayo*	
										64% (7/11)	**27%** (3/11)	16 ± 17	10 ± 12
Alexander [178]	Adultos	21	PF	1760 µg	6 semanas	21	Placebo		6 semanas	*>90% reducción eosinófilos*		*Cuestionario Disfagia Mayo*	
										62% (13/21)	**0%** (0/21)	**43%** (9/21)	**28%** (6/21)
Moawad F [128]	Adultos	21	PF	880 µg	8 semanas	21	Esomeprazol	40 mg	8 semanas	*< 7 eosinófilos/cga*		*Cuestionario Disfagia Mayo*	
										19% (4 /21)	**33%** (7/21)	12 ± 16	1,4 ± 4,5
Butz[173]	Ambos	28	PF	1760 µg	12 semanas	14	Placebo		12 semanas	*< 1 eosinófilo/cga*		No hay diferencias	
										53% (15/28)	**0%** (0/14)		

Tabla 6: Principales ECA con esteroides tópicos para tratamiento de pacientes con EoE. PF: Propionato de fluticasona; cga: campo gran aumento *(Continuación)*

Estudio	Tipo Pb	Grupo Experimental				Grupo Control				Remisión Histológica		Remisión Clínica	
		N	Fármaco	Dosis	Duración	N	Fármaco	Dosis	Duración	Experimental	Control	Experimental	Control
Gupta[179]	Niños	17 19 17	Budesonida viscosa	Baja (0.35 – 0.5 mg) Media (1,4 – 2 mg) Alta (2,8 – 4 mg)	12 semanas	18	Placebo		12 semanas	*≤ 1 eosinófilo/cga* **12%** (2/17) **42%** (8/19) **76%** (13/17)	**0%**	*Score Disfagia* **18%** (3/17) **32%** (6/19) **18%** (3/17)	33% (6/18)
Miehlke[180]	Adultos	19 19 19	Budesonida viscosa Budesonida tabletas	2 mg 2 mg 4 mg	2 semanas	19	Placebo		2 semanas	*< 15 eosinófilo/cga* **94,7%** (18/19) **100%** (19/19) **94,7%** (18/19)	**0%**	*Score Disfagia* 4,5 ± 1,8 4,9 ± 1,4 4,2 ± 2,1	4,7 ± 1,9
Dellon E[181]	Niños y Adultos	51	Suspensión budesonida oral	4 mg	12 semanas	42	Placebo		12 semanas	*< 6 eosinófilos/cga* **39%** (19/49)	**3%** (1/38)	*Score Disfagia* 15 ± 16,9	21,5 ± 16

Las dosis de propionato de fluticasona recomendadas para el tratamiento de la EoE en niños varían de 880 a 1760 µgr para la inducción de la remisión y de 440 a 880 µgr para el mantenimiento de ésta. En el caso de los adultos, las dosis recomendadas son de 1760 µgr para la inducción y de 880 a 1760 µgr para el mantenimiento. En el caso de la budesonida, las dosis recomendadas en niños para la inducción de la remisión son de 1 a 2 mg/día, y 1 mg/día para el mantenimiento; en adultos las dosis recomendadas de budesonida oscilan entre 2 y 4 mg/día para la inducción y 2 mg/día para el mantenimiento de la remisión[1].

Hasta el año 2018 no hemos dispuesto de una formulación de corticoides aprobada específicamente para el tratamiento de la EoE y el uso de estos fármacos se realizaba fuera de ficha técnica, empleando esteroides adaptados de otras indicaciones clínicas.

d) Fármacos antialérgicos

Numerosos fármacos antialérgicos han sido ensayados en el tratamiento de la EoE, pero ni cromoglicato de sodio, ni los antihistamínicos han mostrado ser efectivos para lograr la remisión ni la mejora clínica de la EoE[70]. Los antileucotrienos tampoco parecen ser efectivos para inducir remisión ni para mantener la remisión de la enfermedad[182,183]. También ha sido ensayada una molécula antagonista de CRTH2 (molécula homóloga del receptor quimioatrayente expresado en células Th2) que resultó igualmente inefectiva en la normalización del esófago[184].

e) Azatioptina/6 mercaptopurina

Al ser la EoE una enfermedad mediada inmunológicamente, los fármacos inmunomodulares tiopurínicos azatiopina y 6-mercaptopurina han sido testados en el manejo de los pacientes con EoE. Hasta la fecha, la experiencia con estos tratamientos para inducir la remisión de la enfermedad ha sido escasa, mostrándose eficaz en una pequeña serie de pacientes[185]. Debido a la falta de estudios prospectivos con mayor tamaño muestral y a que existen tratamientos alternativos muy eficaces para el manejo de la enfermedad con un mejor perfil de seguridad, el uso de inmunomoduladores no está aconsejado.

f) Agentes biológicos (anticuerpos monoclonales)

En los últimos años y coincidiendo con el mayor conocimiento de las bases moleculares y celulares de la enfermedad, se ha postulado su tratamiento mediante anticuerpos dirigidos a bloquear la acción de ciertas moléculas con una función potencial en la EoE. El primero en utilizarse fue Mepolizumab, un anticuerpo monoclonal anti-IL-5, que resultó ineficaz para lograr la remisión clínica o sintomática de la EoE, al igual que su homólogo reslizumab. Este tratamiento únicamente consiguió una modesta reducción de la densidad de eosinófilos en el esófago[186–188]. Posteriormente, otros agentes anti-IL13, anti-TNF-alfa y anti-IgE han sido ensayados en pacientes con EoE, dirigidos a potenciales dianas terapéuticas. Ni omalizumab (anti IgE), ni Infliximab (anti TNF-alfa) resultaron eficaces en la remisión de los síntomas ni de la remisión histología[189–191]. Por su parte, QAX576 (molécula anti IL-13) mostró no tener efectos sobre los síntomas pero si conseguir una modesta reducción en la eosinofília esofágica y en la inhibición del transcriptoma especifico de EoE[192]. Futuros desarrollos dirigidos a bloquear esta última diana deberán mostrar su potencial en los próximos años.

5.3.- Tratamiento mediante dilatación endoscópica

Debido a la naturaleza crónica de la enfermedad y a su componente fibrosante del esófago que conduce a reducir el calibre del esófago, la dilatación con balones hidroneumáticos, bujías o dilatadores rígidos se ha empleado para el tratamiento de la EoE. Hasta la fecha la dilatación constituye el único tratamiento endoscópico disponible y aunque no posee efecto alguno sobre la inflamación de la mucosa esofágica ni sobre los fenómenos de remodelación, proporciona una mejoría sintomática inmediata en la gran mayoría de los pacientes (95% de mejoría clínica; IC95%: 90 – 98%)[193]. La dilatación esofágica se ha demostrado ser segura en los pacientes con EoE con escasas complicaciones, entre las que destacan la hospitalización (0,67%), la hemorragia (0,05%) y la perforación (0,38%), sin ningún caso de muerte comunicada, según un meta-análisis y su reciente actualización, que incluyen 27 estudios con más de 1800 dilataciones practicadas[193,194]. **(Tabla 7).**

En todo caso, la dilatación esofágica no debe utilizarse como el único tratamiento para la EoE, debiendo acompañarse siempre de un tratamiento farmacológico o dietético eficaz para conseguir una remisión histológica de la enfermedad.

Tabla 7: Principales estudios realizados con dilataciones para el tratamiento de pacientes con EoE con medición de mejoría clínica.

Estudio	N	Tipo Población	Tipo Dilatación	% Mejoría Clínica
Vasilopoulos S[195]	4	Adultos	Savary	**100%**
Croese J[196]	17	Adultos	Celestin	**94%**
Potter JW[138]	13	Adultos	Savary & TTS	**54%**
Cantu P[197]	2	Adultos	Celestin	**100%**
Pasha SF[198]	18	Adultos	Savary & Maloney	**85%**
Rajagopalan J[199]	1	Adultos	Savary	**100%**
Bohm M[200]	9	Adultos	Savary & Maloney	**80%**
Enns R[201]	15	Adultos	-	**80%**
Robles-Medrana C[202]	4	Niños	TTS	**100%**
Schoepfer AM[203]	207	Adultos	Savary & TTS	**93%**
Lenglinger J[204]	1	Adultos	Esoflip	**100%**
Lipka S[205]	13	Adultos	Savary & Maloney & TTS	**100%**
Seeger K[206]	1	Adultos	TTS	**100%**
Kavitt RT[207]	17	Adultos	Maloney	**94%**
Menard-Katcher C[208]	40	Niños	Maloney & TTS	**86%**
Al-Husani A[209]	10	Niños	Savary	**100%**
Runge TM[210]	164	Niños y Adultos	Savary & TTS	**85%**

6.- Algoritmo de tratamiento de la EoE

Teniendo en cuenta lo antes mencionado, la guía clínica más reciente de la enfermedad ha propuesto un algoritmo terapéutico para el manejo efectivo de los pacientes con EoE[1] (**Figura 3**). Brevemente, cualquier terapia con potencial anti-inflamatorio constituye una primera línea terapéutica: IBPs, corticoides tópicos deglutidos o dieta de eliminación. La elección de una u otra opción será individual para cada paciente, considerando su estilo de vida, preferencias, edad, recursos, calidad de vida y disponibilidad para el tratamiento. No se recomienda combinar estos tratamientos.

En el caso de alcanzar la remisión clínica e histológica, el paciente podrá seguir con este tratamiento a largo plazo como manteniendo, o considerar un cambio a otra alternativa que le resulte más conveniente. Si el primer tratamiento elegido no conduce a la remisión se puede cambiar a alguna de las otras dos opciones. En caso de persistencia de síntomas pese a lograr remisión histológica probablemente se deba a una estenosis o reducción del calibre del esófago, debiendo considerar la dilatación endoscópica. Sólo si no se consigue la remisión de la enfermedad con ningún tratamiento anti-inflamatorio propuesto deberá remitirse al paciente a un centro especializado o plantear realizar dietas elementales.

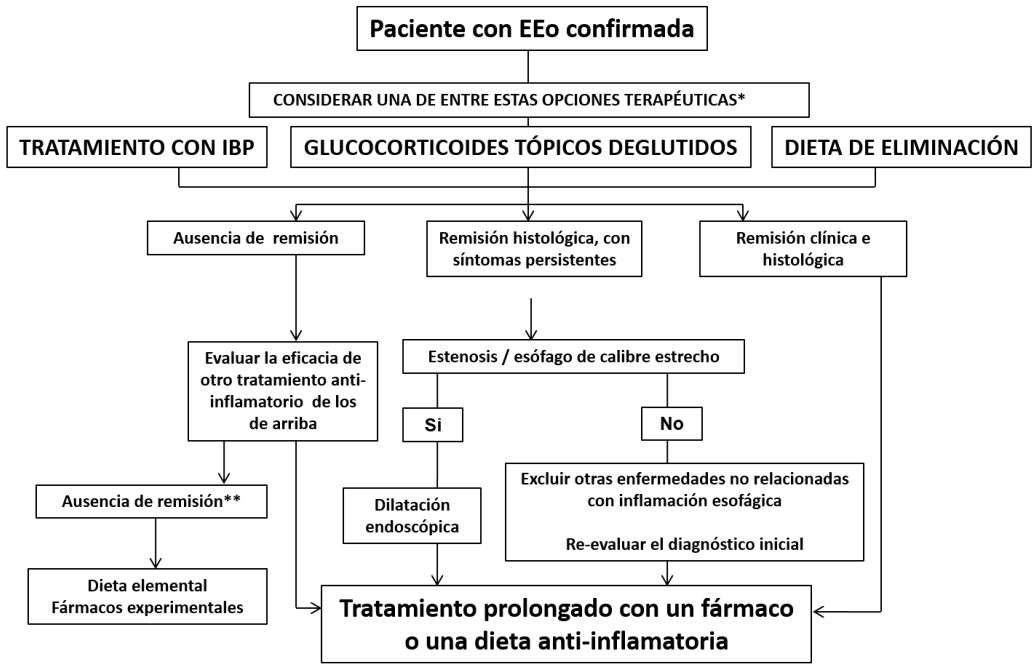

Figura 3: Algoritmo terapéutico para el manejo de la EoE[1].

7.- Factores de Riesgo y Predisposición genética

Uno de los primeros factores de riesgo identificados para la EoE fue el sexo masculino, ya que la gran mayoría de los estudios reportan que los varones presentan mayor frecuencia de EoE que las mujeres. Este riesgo fue cuantificado en más de 2 veces en una revisión sistemática[22]. Por esta razón se ha postulado que los cromosomas sexuales estarían implicados en esta predominancia de la enfermedad entre los varones y en los mecanismos de herencia de la enfermedad (por ejemplo, dos cadenas proteicas del receptor de la IL-13, molécula con un papel fundamental en la fisiopatología de la enfermedad, se encuentran codificadas en el cromosoma X). Además, el gen del receptor de la linfopoyetina estromal tímica (TSLP) está codificado en los cromosomas sexuales, y se ha descrito un polimorfismo de un solo nucleótido (SNP) en el gen de esta citoquina que podría constituir un factor de riesgo para la enfermedad[211].

Otros factores genéticos asociados al riesgo de desarrollo de la EoE incluyen un SNP localizado en una región no transcrita (3'UTR) del gen de eotaxina 3, que podría estar implicado en la estabilización del RNAm de eotaxina-3 y por tanto constituir un factor que predispone a padecer la enfermedad[212]. Sin embargo estudios más recientes destacan también el papel de los factores ambientales para el desarrollo de la EoE[213].

La asociación familiar de casos ha sido ampliamente descrita en la literatura científica, con coincidencia de EoE principalmente entre hermanos. Para otros grados de parentesco también existe mayor prevalencia de casos de EoE en familiares de pacientes con EoE, que también apoya una base genética para la enfermedad. De hecho, hasta un 7 - 8% de los casos con EoE tienen algún otro familiar afectado por la enfermedad[14,214], sugiriendo mecanismos genéticos complejos de herencia. Un reciente estudio ha cuantificado el riesgo de padecer EoE en función del grado de parentesco. Mientras la prevalencia de EoE en la población general (o su riesgo general) se estimaba en un 0.05% (1/2,000 habitantes), ésta aumentó hasta 2,4% en hermanos, un 22% en gemelos dicigóticos y 41% en gemelos monocigóticos. El hecho de que estos últimos compartan un 100% de su identidad genética sugiere la influencia relevante de factores ambientales en el origen de la enfermedad[213]. Debido a que los gemelos dicigóticos y los hermanos nacidos sucesivamente poseen la misma relación genética, los autores utilizaron esta diferencia para determinar que los factores ambientales

contribuyen en un 81% a la variación fenotípica en el desarrollo de EoE. Por este motivo, la contribución de las variantes de riesgo genético representaría únicamente un 15% de la variación fenotípica del riesgo de enfermedad. Más recientemente, un estudio realizado en Utah ha confirmado que el riesgo de padecer EoE aumenta entre los familiares de primer grado (OR 7,19), aunque el aumento de riesgo entre los familiares de segundo grado y los primos hermanos también resulto significativo (OR 1,99 y 1,03, respectivamente. Este estudio también observó un aumento de riesgo entre los cónyuges de pacientes con EoE (OR, 2,86), un dato que apoya la importancia de la exposición ambiental para el desarrollo de la enfermedad.

La atopia ha sido considerada históricamente un factor de riesgo relevante para el desarrollo de EoE, al presentar los pacientes con EoE una mayor frecuencia de rinitis, asma y eczema. Una revisión sistemática ha cuantificado esta mayor frecuencia en los pacientes con EoE respecto a la población general: la presencia de asma mostró un OR de 3 [IC95%: 2 – 4.6]) respecto a los controles sin enfermedad, la rinitis alérgica un OR de 5.01 [IC95%: 2.9 – 8.9]) y el eczema un OR de 2.9 [IC95%; 1.9 – 4.3]). Sin embargo, hasta la fecha no ha podido demostrarse que presentar atopia predisponga a sufrir posteriormente EoE[215], aunque recientemente un estudio sugiere que la EoE es una manifestación tardía de la marcha alérgica o de la historia natural de las condiciones alérgicas a medida que se desarrollan durante la infancia[216].

La EoE también ha sido relacionada con otras patologías como la enfermedad celiaca, síndromes hipereosinofílicos, enfermedad inflamatoria intestinal, atresia esofágica y otros trastornos del tejido conectivo, aunque no se ha demostrado una asociación casual entre ninguna de estas patologías y la EoE[217]. De hecho la asociación más estudiada ha sido con la enfermedad celiaca y al menso dos revisiones sistemáticas han demostrado que no hay suficiente evidencia científica para confirmar dicha asociación[218,219].

8.- Aspectos genéticos e inmunopatogénicos de la EoE

Diversos estudios desarrollados especialmente en la última década han comenzado a delinear los mecanismos moleculares y celulares implicados en el origen de la EoE, sin que hasta la fecha dispongamos de una explicación completa sobre el origen de la enfermedad. En todo caso, y al igual que ocurre con otras enfermedades inmunológicas, la EoE resulta de la interacción de una predisposición genética individual, la interacción con factores ambientales y la exposición a antígenos de la dieta.

8.1.- Aspectos moleculares

La EoE se ha identificado con una respuesta inmunológica de tipo Th2 mediada por linfocitos T CD4+ y llevada a cabo predominantemente por citoquinas como IL-4, IL-5, IL-9 e IL-13.

El perfil de expresión génica específico de los pacientes con EoE fue descrito por primera vez por Blanchard y colaboradores[212]. Los pacientes con EoE presentan un transcriptoma esofágico específico caracterizado por cambios en la expresión de 574 genes, que se corresponden aproximadamente al 1% del genoma humano. De entre ellos, 344 genes se encuentran sobreexpresados y 230 están inhibidos en relación a los sujetos sin la enfermedad. El gen con los mayores cambios en su expresión es el que codifica para eotaxina 3 (que aparece sobreexpresado en más de 50 veces), una quimocina relacionada con la quimiotasis de eosinófilos hacia los tejidos. Otros genes sobreexpresados incluyen varios relacionados con el mantenimiento de la función de la barrera epitelial, la síntesis y la maduración de inmunoglobulinas, y genes relacionados con la función de los mastocitos. Además, los cambios en la expresión génica revierten tras el tratamiento con esteroides tópicos. Investigaciones posteriores han expandido el transcriptoma específico de la EoE mediante secuenciación masiva del RNA[220], obteniéndose 1.607 transcritos con una expresión alterada en los pacientes con EoE, de los cuales 1.085 estaban sobre-expresados y 511 estaban inhibidos. Estos estudios muestran que tanto las eotaxinas como otras interleuquinas, entre las que destacan la IL-5 e IL-13, desempeñan un papel central en la enfermedad. Es bien conocido que la IL-5 está sobreexpresada en modelos experimentales de EoE en ratones[221,222]

y en pacientes con EoE y que IL-5 promueve la proliferación, maduración y la supervivencia de los eosinófilos y facilita su migración desde la médula ósea hasta la sangre. Por su lado, IL-13 induce la expresión de la eotaxina-3 en las células epiteliales del esófago mediante el factor de transcripción nuclear STAT6. La IL13 también contribuye a la hiperalgesia esofágica mediante la inhibición de la expresión de filagrina e involucrina, altera la integridad de la barrera epitelial reduciendo las moléculas de adhesión como desmoglina 1[223], lo que incrementa la permeabilidad de la membrana[224]. La disfunción del epitelio puede facilitar la penetración de alérgenos no degradados que perpetuán la respuesta inflamatoria.

8.2.- Aspectos celulares

El infiltrado inflamatorio que caracteriza la EoE está constituido por varios tipos celulares que contribuyen a la inmunopatología de la enfermedad:

a) Eosinófilos

Los eosinófilos son las células más características entre las implicados en la EoE, constituyendo su densidad en el epitelio esofágico el aspecto histológico definitorio de la enfermedad, a la vez que su desaparición el elemento definitorio de la eficacia del tratamiento. Los eosinófilos son granulocitos derivados de la médula ósea cuyos gránulos citoplasmáticos contienen pigmentos básicos que se unen a colorantes ácidos como la eosina. Los eosinófilos poseen funciones proinflamatorias y su función principal es la protección frente a parásitos, participando también como mediadores en las reacciones alérgicas. Son células capaces de causar daños tisulares a través de las proteínas citotóxicas contenidos en sus gránulos; también pueden liberar mediadores inflamatorios que activan a las células epiteliales, los linfocitos T y provocar respuestas inmunes antígeno-específicas actuando como células presentadoras de antígenos[225].

Las principales proteínas citotóxicas del eosinófilo son la proteína mayor básica (MBP), proteína catiónica del eosinófilo (ECO), peroxidasa eosinofílica (EPO) y neurotoxina derivada del eosinófilo (EDN). Los eosinófilos también utilizan moléculas de adhesión y factores quimiotácticos para dirigirse a los tejidos del torrente sanguíneo. La MBP incrementa la reactividad del músculo liso y favorece la degranulación de mastocitos y basófilos. La acumulación, crecimiento, diferenciación, maduración y activación de los eosinófilos está mediada principalmente por las eotaxinas y por la IL-5. Aunque no es descartable la actuación de otros mecanismos implicados, puesto que la utilización de un anti-IL-5, como el Mepoluzimab, tiene un escaso éxito en lograr la remisión de la enfermedad[186,187].

Los eosinófilos no están presentes en el esófago humano en condiciones normales y habitualmente no infiltran el epitelio.

b) Mastocitos

Junto con los eosinófilos, los mastocitos también se encuentran presentes en el infiltrado inflamatorio específico de la EoE, aunque en menor densidad que los eosinófilos. Se ha observado una correlación entre las densidades de mastocitos y eosinófilos en el epitelio esofágico[226], y ambas se reducen después del tratamiento con corticoides[168] y dieta. Además de una mayor densidad de mastocitos en pacientes adultos[67,227] como niños con EoE[69,228,229] en relación a los pacientes sanos, se han demostrado signos de activación mastocitaria en los pacientes con EoE.

Los mastocitos son células mesenquimales derivadas de células mieloides de la médula ósea, que a diferencia de otras células mieloides completan su diferenciación y maduración en los tejidos periféricos. Su crecimiento y diferenciación están influenciados por las citoquinas IL-3, IL-4, IL-9, IL-10, *stem cell factor* (SCF), factores de crecimiento, prostagladinas y la interacción con algunas moléculas de adhesión. En humanos SCF es la principal citoquina responsable de la maduración activación y quimiotaxis de los mastocitos. La maduración final de mastocitos está condicionada por la interacción con el microambiente del tejido donde se

encuentren y es muy activa si existe inflamación. Los mastocitos están ampliamente distribuidos en todos los tejidos conectivos y forman parte del sistema inmune innato contra las bacterias y parásitos; también desempeñan un papel importante en enfermedades alérgicas como la EoE[69,228–230].

Los mastocitos humanos se dividen en dos grupos según el contenido de sus orgánulos, los mastocitos con triptasa (MC_T) o los mastocitos con triptasa y quimasa (MC_{TC}). Generalmente los MC_T se localizan en los tejidos mucosos, mientras que los MC_{TC} predominan en tejidos conectivos, aunque también pueden ser encontrados en la submucosa y a veces en las muscularis propia de los órganos del tubo digestivo[231,232]. Esta diversidad fenotípica implica también la regulación diferencial de la expresión génica de citoquinas y se asocia con diferencias funcionales. Así los MC_{TC} son respondedores a estímulos no mediados por IgE que comprenden desde la activación por parte de los TLRs hasta mecanismos no inmunológicos[233–235].

El transcriptoma específico de la EoE también muestran genes específicos de mastocitos sobreexpresados, como los de triptasa y carboxipeptidasa, por lo que estas células también desempeñan un papel importante en la enfermedad[69,230]. La activación de estos mastocitos produce la liberación de diversas moléculas como TGF-β que está implicado en procesos de remodelación fibrosa del tejido subepitelial del esófago[47]. Sin embargo, la naturaleza de estos mastocitos, su relación con las manifestaciones clínicas y su función fisiopatológica en la EoE no habían sido todavía aclaradas antes de nuestro trabajo, así como el efecto de los diversos tratamientos para la EoE sobre la densidad de mastocitos y la expresión génica de sus proteasas.

El mecanismo de activación de los mastocitos en la EoE aún no ha sido aclarado, pero además del bien estudiado entrecruzamiento de IgE sobre los receptores de alta afinidad (FCeRI) de su superficie que participan en reacciones anafilácticas, en esta enfermedad parecen existir otros mecanismos alternativos. Los mastocitos también pueden ser activados a través de vías menos

conocidas, como a través de TLRs o por vías no inmunológicas[233]. Hasta la fecha estos mecanismos no han sido estudiados en la EoE y ningún trabajo ha valorado la función que los TLRs pudieran desempeñar en la mucosa esofágica de los pacientes con EoE a nivel de los mastocitos y las células epiteliales.

c) Células B

Un estudio ha revelado una mayor densidad de linfocitos B en el epitelio esofágico de pacientes con EoE[66], pero no hay estudios que analicen el papel exacto de estos linfocitos B en la enfermedad.

d) Células epiteliales

La función central que desempeñan las células epiteliales en la EoE es motivo de interés creciente y cada vez está mejor definido. El epitelio del tracto gastrointestinal posee funciones en el mantenimiento de la homeostasis, al coordinar la respuesta inmune después de la integración de las señales internas y externas, contribuyendo al mantenimiento del equilibrio entre los componentes inflamatorio y no inflamatorio.

Además las células epiteliales son una de las principales fuentes de eotaxina-3, CXCL16 y TSLP[212], que como ya se ha dicho anteriormente, son moléculas con un papel principal en la EoE. Los eosinófilos, mastocitos y linfocitos infiltrados en el epitelio esofágico podrían liberar mediadores como IL-13 que activarían la expresión de diversos genes como el de eotaxina-3 en las células epiteliales

e) Células iNKT

Las células T asesinas naturales invariantes (iNKT por sus siglas en inglés) son un subtipo de linfocitos T implicados en la respuesta inmune, que responden a glicolípidos y esfingolípido (presentados por CD1d), en lugar de a proteínas presentadas por el receptor de células T (TCR). Los esfingolipidos pueden encontrarse en diversos alimentos, como leche y huevos. Se ha observado que los esfingolipidos de la leche pueden activar las iNKT en niños con EoE[236], y que ratones deficientes para CD1d están protegidos frente al desarrollo de la enfermedad[237].

Las células iNKT son reclutadas por acción de CXCL16 producida por las células epiteliales y dendríticas, y una vez estimuladas promueven una rápida respuesta Th2 característica de la EoE, con un aumento en la expresión de las principales citoquinas implicadas en la enfermedad (IL-4, IL-5, IL-13) y eotaxinas[237]. Se ha observado un aumento en la densidad y en la actividad de células iNKT en el esófago de niños con EoE, en los que la posterior eliminación de los alérgenos de la dieta disminuyó los marcadores de actividad[238], como prueba del papel importante de estas células en la enfermedad.

f) Células dendríticas CD1a+

El esófago contiene células dendríticas CD1a+ que también pueden actuar como link entre el sistema inmune innato y adaptativo[67].

8.3.- Factores ambientales

Los factores ambientales cada vez son más reconocidos y estudiados en la EoE. El primer estudio que puso de relevancia su importancia fue publicado por Jensen y colaboradores en el año 2013[32]. Junto con otras investigaciones posteriores[33,239,240] este estudio mostró que los factores prenatales y las exposiciones tempranas (durante los primeros años de vida) parecen ser esenciales para determinar el riesgo de EoE. Entre ellas, la exposición a antibióticos durante la infancia, el parto por cesárea, la lactancia materna no exclusiva y el parto prematuro estaban todos asociados al mayor riesgo de padecer EoE. Todos los factores descritos son capaces de inducir cambios en la microbiota del tubo digestivo en general y esofágica en particular, dando lugar a la hipótesis de que cambios composición de la microbiota (tanto en el número como en la proporción de sus componentes) desencadenan el desarrollo de EoE[241]. En contraste, la convivencia con mascotas de pelo largo durante la infancia se ha identificado como un factor protector frente a la EoE[33]. La densidad de población (rural *versus* urbana) y la exposición aeroalergénica también han sido descritos como posibles factores de riesgo[31,242].

La interacción entre genes y factores ambientales en el desarrollo de la EoE ha sido evaluada sólo muy recientemente en un estudio preliminar que analizó las interacciones entre los polimorfismos que predisponen a la EoE (en TSLP, LOC283710/KLF13, CAPN14, CCL26 y TGFβ) y las exposiciones durante las primeras etapas de la vida. Se demostraron las interacciones entre rs6736278 (CAPN14) y la lactancia (p=0.02) y rs17815905 (LOC283710 / KLF13) y el ingreso en una unidad de cuidados intensivos neonatales (p=0.02)[32]. Además, los autores encontraron que la lactancia materna tenía un fuerte efecto protector en aquellos pacientes con el genotipo de susceptibilidad en el gen CAPN14, lo que sugiere por primera vez en la literatura que el riesgo de enfermedad por EoE podría ser modificable en sujetos con ciertas exposiciones ambientales y variantes genéticas.

En resumen, la EoE se produce como consecuencia de una interacción de causas genéticas, con factores moleculares, celulares y ambientales. Es decir una exposición temprana a determinados factores ambientales (que probablemente modifican el microbioma esofágico / gastrointestinal) en sujetos genéticamente susceptibles parece determinar el desarrollo de EoE.

Por último, en los últimos años se ha comenzado a estudiar la influencia de los cambios epigenéticos sobre el desarrollo de la EoE; estudios preliminares han observado una expresión diferencial en 32 micro-RNAs en pacientes con EoE, de los cuales 21 se encuentran sobre-expresados y 11 se encuentran inhibidos.

9.- Modelo explicativo integrado

Un modelo genético molecular explicativo de la enfermedad fue propuesto por el Dr Lucendo[243], donde las células epiteliales y dendríticas expresarían CXCL16 que producirían el reclutamiento y activación de las células iNKT que a su vez constituirían la fuente primera de citoquinas de tipo Th2 como IL-4, IL-5 e IL-13, entre otras. Actuando sobre las células epiteliales la IL-13 alteraría la función de barrera del epitelio produciéndose un aumento de la permeabilidad y la penetración de alérgenos de la dieta. Por su parte, IL-5 ejercería un efecto a distancia sobre la médula ósea fomentando la producción y liberación de eosinófilos hacia el torrente sanguíneo. Las células epiteliales también son la fuente principal de eotaxina-3 promoviendo la migración de los eosinófilos desde la sangre hacia el esófago, que

una vez activados por IL-5 degranularían su contenido. Los mastocitos también son reclutados hacia el esófago donde maduran, se activan y, junto con los eosinófilos liberarían TGF-β y otras moléculas profibrogénicas como CCL18 o el FGF-9[243], responsables de la remodelación fibrosa del esófago. Su efecto sobre los fibroblastos del subepitelio conduciría, con el paso del tiempo, al depósito de colágeno, la fibrosis del esófago y la formación de estenosis. Esta fibrosis puede ser revertida mediante tratamientos eficaces capaces de inducir la remisión de la inflamación[243].

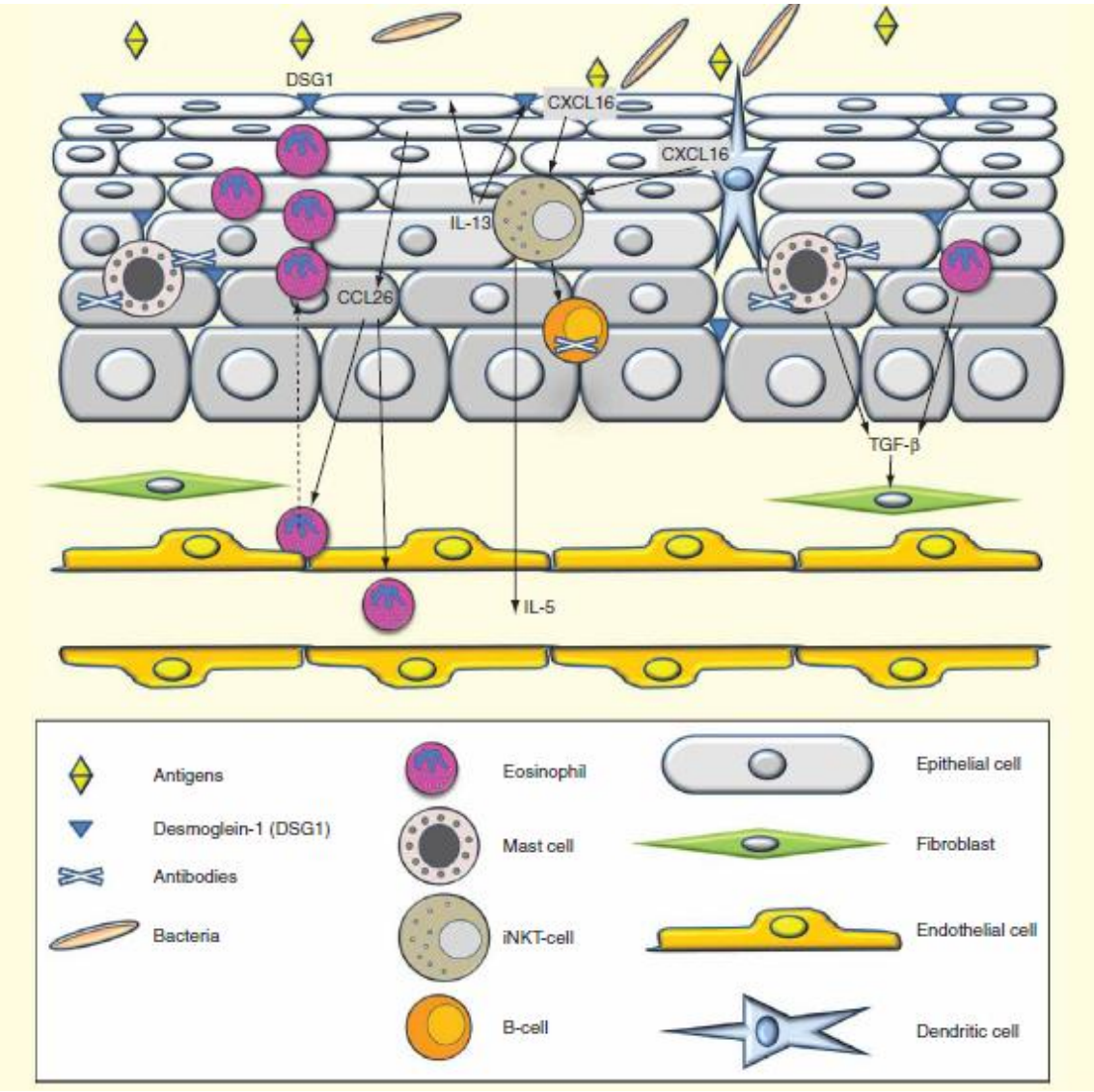

Figura 4: Modelo genético explicativo integrado de la EoE (modificado de *Lucendo et al*)

10.- Sistemas inmune adaptativo e innato y función de los TLRs

10.1.- Sistema inmune innato

El sistema inmune innato reconoce y responde a ofensas ambientales y patógenas sin la necesidad de una respuesta antígeno-específica mediada por inmunoglobulinas y, por tanto, sin conferir inmunidad a largo plazo. Existen recientes evidencias de su potencial papel en la EoE.

Las principales funciones del sistema inmune innato incluyen el reclutamiento de células inmunes hacia los sitios de infección y de inflamación mediante la acción de citoquinas, la activación del sistema del complemento, la identificación y eliminación de sustancias extrañas presentes en los tejidos y la activación del sistema inmune adaptativo mediante la presentación de antígenos.

Los mecanismos implicados en la respuesta inmune innata incluyen, entre otros, los mecanismos de barrera, activación de determinadas células en el tejido y secreciones. La inflamación es una de las primeras respuestas del sistema inmunitario, que permite establecer una barrera física contra la propagación de las agresiones, como la infección, y para promover la recuperación de los tejidos dañados.

Los mastocitos son una de las células implicadas en la acción del sistema inmune innato (al que también contribuyen las células *natural killers*, eosinófilos, basófilos, macrófagos, neutrófilos y células dendríticas). Los mastocitos se localizan en la interfase entre el huésped y el ambiente externo, estableciendo contacto con patógenos y antígenos, y activando la respuesta inmune. Los mastocitos poseen una gran variedad de receptores para poder interactuar con numerosos patógenos y/o antígenos, entre los que destacan los receptores del complemento (CR3, CR4 y CR5) que reconocen factores del complemento sobre la pared bacteriana, receptores Fc para la fracción constante de las inmunoglobulinas (FCγRI; FcγRII, FCγRIII, FCεRI) y los TLRs. El sistema inmunitario innato está íntimamente ligado al sistema inmunitario adaptativo. Las células de sistema inmunitario innato, procesan los antígenos y los presentan a los linfocitos.

10.2.- Sistema inmunitario adaptativo

Al contrario que el sistema inmunitario innato, el sistema inmune adaptativo está dirigido específicamente contra el agente infeccioso o antígeno responsable de la agresión. El sistema inmunitario se adapta con el tiempo para reconocer patógenos específicos de manera más eficaz, generando una memoria inmunitaria.

En este tipo de respuesta se encuentran implicados principalmente los linfocitos T y los linfocitos B cuya respuesta es específica para cada antígeno. La respuesta inmune específica puede ser de dos tipos: humoral, cuando los elementos implicados son los productos de los linfocitos B madurados hacia células plasmáticas, y celular, cuando participan prioritariamente los linfocitos T, tanto colaboradores como citotóxicos. En la inmunidad específica también se implican otras células, como los mastocitos, que son capaces de fagocitar, procesar y captar antígenos, modular el crecimiento y reclutamiento de linfocitos y producir inmunoglobulina, así como presentar antígenos por mecanismos dependientes de MCH de clase I y II, y modular la migración, maduración y activación de las células dendríticas.

Los mastocitos pueden interaccionar con linfocitos B y T, pueden secretar citoquinas como TBF-α, IL-1β, IL-4, IL-5, IL-8 e IL-13, que activan a linfocitos y macrófagos. Los mastocitos no solo actúan como células efectoras proinflamatorias en las respuestas inmunes, sino que también contribuyen a su inicio y regulación.

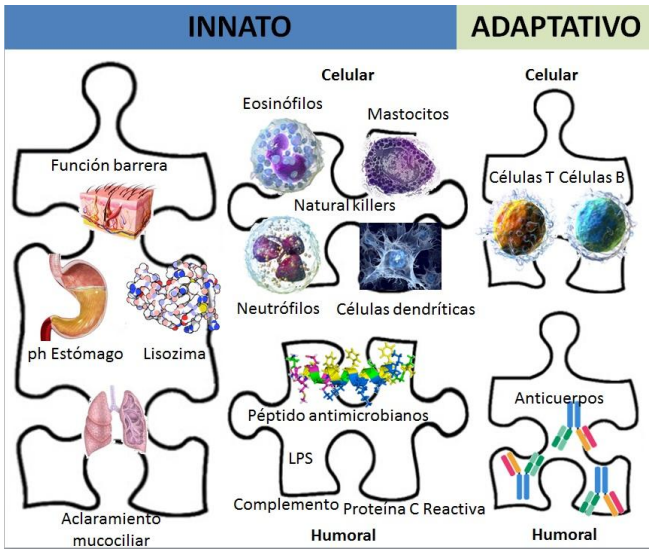

Figura 5: Inter-relación entre el sistema inmune innato y adaptativo

10.3.- TLRs y mastocitos, que constituyen el nexo de unión entre los sistemas inmune innato y adaptativo.

Una de las propiedades esenciales del sistema inmune de los mamíferos es la capacidad de producir una respuesta ante diferentes patógenos y a la vez mantener una tolerancia frente a los propios. En los últimos años ha aumentado exponencialmente el conocimiento acerca de los componentes moleculares y de las funciones del sistema inmune innato en la defensa del huésped. El reconocimiento de la mayoría de los microorganismos se realiza por varias familias de receptores. Estos receptores de reconocimiento de patrones (PRR) reconocen patrones moleculares asociados a patógenos (PAMP) y activan células de la vía innata. Dentro de este grupo de receptores se encuentran los TLRs[244], que son una familia de receptores transmembrana y/o intracelulares responsables, entre otras funciones, del reconocimiento de patógenos que están implicados en la respuesta inflamatoria, siendo un nexo de unión entre el sistema inmune innato y el sistema inmune adaptativo.

La activación y maduración de las células presentadoras de antígenos y de las células T reguladoras dependen, entre otras, de las vías de señalización mediadas por TLRs. Una de las vías de activación de los mastocitos depende de la señalización mediada por TLRs. De esta forma, los TLRs podrían influir decisivamente en la homeostasis inmune de la mucosa esofágica y en la pérdida de la tolerancia inmunológica que se produce en la EoE.

En el hombre, se conocen 11 tipos distintos de TLRs (numerados desde TLR-1 hasta TLR-11) que son capaces de distinguir tipos distintos de patógenos (mediante el reconocimiento de PAMP), cada uno, poniendo en marcha una respuesta inmunitaria inflamatoria específica **(Tabla 8)**. La mayoría de los TLRs se encuentran ampliamente distribuidos en diferentes tipos celulares del sistema inmune incluyendo células dendríticas, macrófagos, células *natural killer*, mastocitos, neutrófilos y linfocitos T y B; aunque también se encuentran en células que no forman parte directa del sistema inmune, como fibrobastos, células epiteliales y queratinocitos[245]. Tras su estimulación generan una transducción de señales intracelulares a través de las MAP-Kinasas y NF-κβ, dando lugar a la expresión o inhibición de determinas genes relacionados con la respuesta inmune inflamatoria

como los que codifican para citoquinas pro-inflamatorias, especies reactivas de oxigeno, o mediadores de citotoxicidad directa[246,247]. Todo esto podría contribuir a una cronificación de la inflamación característica de la enfermedad. A su vez, la inflamación y la lesión tisular provocan la liberación de ligandos endógenos endógenos de los TLR, conocidos como patrones moleculares asociados al daño (DAMP), que son estímulos inflamatorios potentes en rápido crecimiento. Los DAMP actúan de manera autocrina, alertando al huésped del daño, pero también pueden amplificar la inflamación que conduce a un mayor daño tisular. Además, la señalización mediada por TLRs en las células presentadoras de antígenos constituye un nexo de unión entre la respuesta innata y la respuesta adaptativa[248].

Un estudio reciente ha demostrado por primera vez la expresión de TLRs en células epiteliales del esófago[249], pero hasta fechas más recientes ninguna investigación había valorado la función de los TLRs en la EoE, aún siendo el esófago un órgano expuesto a distintos antígenos de origen microbiano y alimentario.

Tabla 8: TLRs descritos en humanos, su ubicación celular y ligandos conocidos.

TLR	Ubicación	Células	Ligandos conocidos	Ubicación ligando
TLR1	Superficie celular	Monocitos, macrófagos, células dendríticas y linfocitos B	Lipopéptidos	Bacterias
TLR2	Superficie celular	Monocitos, macrófagos, células dendríticas y mastocitos	Glicolípidos, lipopéptidos, lipoproteínas, ácido lipoteicoico y otros	Bacterias
TLR3	Intracelular	Células dendríticas y linfocitos B	RNA de doble cadena	Virus
TLR4	Superficie celular	Monocitos, macrófagos, células dendríticas, mastocitos, linfocitos B y células epiteliales	Lipopolisacáridos, fibrinógeno, ácido hialurónico, proteínas y otros	Bacterias
TLR5	Superficie celular	Monocitos, macrófagos, células dendríticas y células epiteliales	Flagelina	Bacterias
TLR6	Superficie celular	Monocitos, macrófagos, mastocitos y linfocitos B	Lipopolipéptidos	Bacterias

Tabla 8: TLRs descritos en humanos, su ubicación celular y ligandos conocidos. *(Continuación)*

TLR	Ubicación	Células	Ligandos conocidos	Ubicación ligando
TLR7	Intracelular	Monocitos, macrófagos, células dendríticas y linfocitos B	RNA cadena simple y otros	Virus
TLR8	Intracelular	Monocitos, macrófagos, células dendríticas y mastocitos	RNA cadena simple y otros	Virus
TLR9	Intracelular	Monocitos, macrófagos, células dendríticas y linfocitos B	Oligonucleótidos, DNA y otros	Bacterias
TLR10	Superficie celular	Monocitos	Hongos	Desconocido
TLR11	Intracelular	Monocitos, macrófagos y células epiteliales	Ácido hialurónico y otros	Bacterias

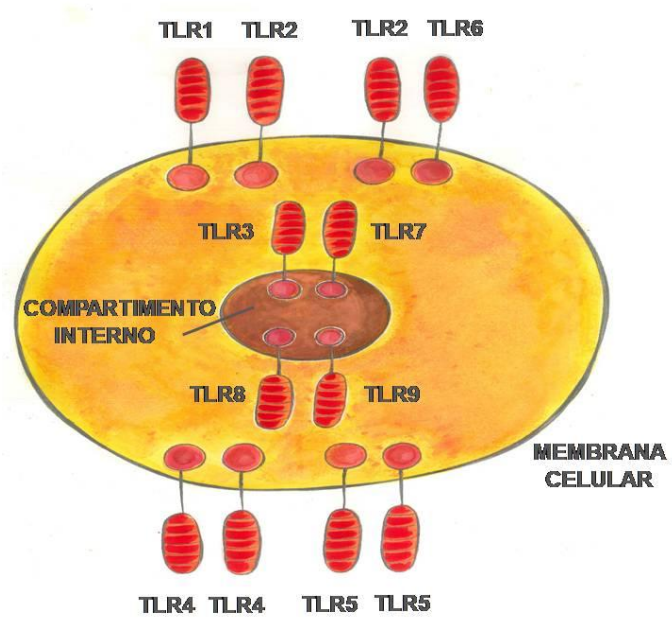

Figura 6: Tipos y localización de los TLRs

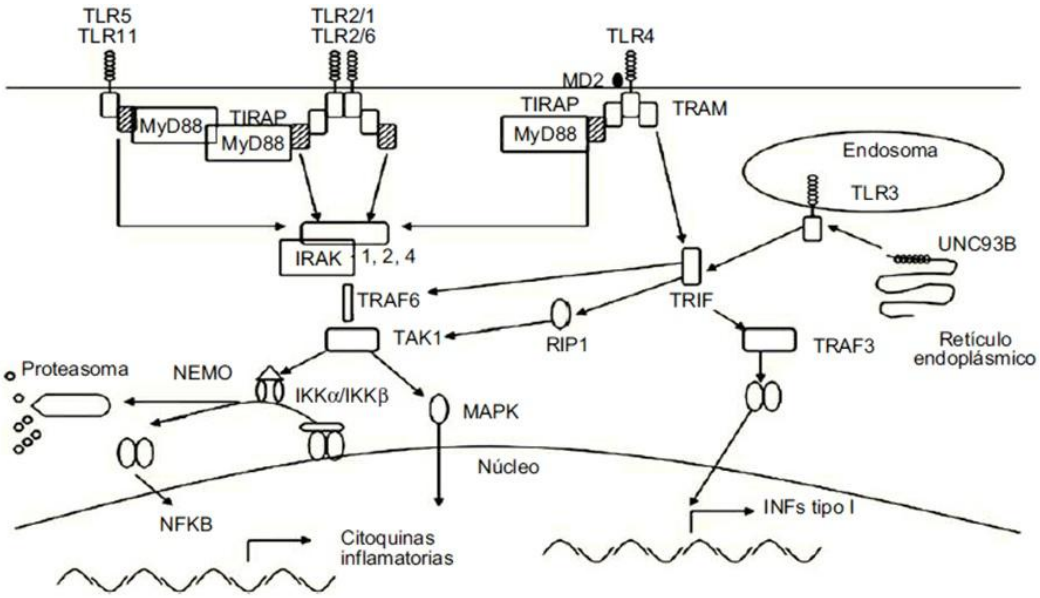

Figura 7: Vías de transducción de señales de los TLRs[248].

Por otro lado, trabajos recientes han comenzado a mostrar los cambios en la composición del microbioma esofágico de niños y adultos con EoE en comparación con controles sin la enfermedad[250,251], mientras que la modificación en la composición de la microbiota causada por el consumo de antibióticos ha sido identificada como un factor de riesgo precoz para el desarrollo de la EoE[33]. Los efectos moduladores de la microbiota sobre la inmunidad innata esofágica y la pérdida de tolerancia derivada de las alteraciones en su interacción constituyen, por tanto, un mecanismo fisiopatológico potencial en el origen de la enfermedad.

En resumen, las interacciones de la microbiota esofágica con los componentes del sistema inmune innato a través de los TLRs y sus vías de señalización y conexión con el sistema inmune adaptativo podrían desempeñar un papel relevante en el origen fisiopatológico de la EoE a múltiples niveles, que requiere ser investigado en profundidad.

HIPÓTESIS

Nuestra hipótesis de trabajo propone que las cifras de incidencia y prevalencia de la EoE, lejos de estabilizarse, continúan en aumento en nuestra área geográfica en pacientes de todas las edades, en concordancia con otros estudios epidemiológicos publicados hasta la fecha. Este hecho implicaría que los mecanismos patogénicos que conducen al desarrollo de la enfermedad continúan actuando y, entre ellos, los relacionados con la función y regulación de los componentes del sistema inmune adaptativo ocuparían una posición central.

En relación con estos mecanismos celulares y moleculares, los mastocitos desarrollarían una función relevante específica y su densidad y estado de activación estaría aumentada en los pacientes con EoE. La expresión de sus proteasas específicas estaría asociada directamente a la actividad clínica de la enfermedad. El tratamiento eficaz de la EoE mediante dietas de eliminación inducirá no solo la reducción de la inflamación eosinofílica del órgano, sino también la densidad de mastocitos en el epitelio esofágico y su estado de activación.

Por último, las pruebas indirectas que relacionan los cambios en la microbiota esofágica con el riesgo de desarrollar EoE nos permite proponer la hipótesis de que la señalización mediada por TLRs desempeña una función potencial en esta enfermedad. Distintos mediadores resultantes de la activación de TLRs estarán aumentados en muestras de esófago de pacientes con EoE activa en comparación con las obtenidas de controles y mostrarán una asociación con los efectores de la activación del sistema inmune innato. La remisión de la enfermedad mediante un tratamiento eficaz normalizará la expresión de la vía de señalización mediada por TLRs, incluyendo aquella de moléculas mediadoras y efectoras.

En conjunto, a partir de nuestros datos y de su integración en el cuerpo de conocimiento actual sobre la etiopatogenia de la EoE, ofrecemos una hipótesis integral sobre la fisiopatología de la enfermedad, que considere la acción de la microbiota esofágica, la función reguladora del epitelio esofágico y la disfunción epitelial como elemento central en el origen y perpetuación de la inflamación, y la participación de los componentes del sistema inmune innato en los fenómenos asociados a la EoE.

OBJETIVOS

Objetivos generales:

Los objetivos generales de este estudio incluyen conocer la epidemiología de la EoE y su evolución en los últimos años en nuestra área geográfica, definir el fenotipo de los mastocitos esofágicos y su asociación con las manifestaciones clínicas de la EoE. Por último pretendemos caracterizar la respuesta innata esofágica mediada por TLRs en la EoE y su regulación a través del tratamiento dietético de la enfermedad.

Objetivos específicos:

1 - Calcular la incidencia y la prevalencia de la EoE de manera global y a lo largo de las diferentes edades en una región central de España y analizar sus tendencias a lo largo del periodo 2006 – 2017.

2 - Analizar la densidad y caracterizar el fenotipo de los mastocitos de la mucosa esofágica en los pacientes adultos con EoE, sus diferencias respecto a los controles sin la enfermedad y su relación con los síntomas.

3 - Cuantificar la expresión génica e identificar la expresión proteica de las proteasas específicas de los mastocitos (quimasa, triptasa y carboxipeptidasa A3) y su actividad biológica en muestras de pacientes con EoE en comparación con controles, y evaluar su reversibilidad mediante tratamiento dietético.

4 - Determinar el nivel de expresión de las principales citoquinas responsables de la atracción y reclutamiento tisular de eosinófilos y mastocitos (CCL11, CCL24, CCL26, SCF, TGF-β), de los principales receptores de éstas células (CCR3 y SCFR), las relaciones entre ellas en muestras de pacientes adultos con EoE, y su asociación con los síntomas.

5 - Evaluar la carga bacteriana y el nivel de expresión diferencial de los RNAm de los principales TLRs 1, 2, 3, 4, 6 y 9 en muestras de mucosa esofágica y duodenal de pacientes adultos con EoE.

6 - Cuantificar el nivel de expresión génica de las principales moléculas adaptadoras, mediadoras (MyD88, NF-κβ, IL-1α, IL-1β, IL-6, IL-8, IL-10 y TNF-α) y efectoras (PER-1, iNOS, GZMA, GZMB) de la vía de señalización de los TLRs en muestras de esófago y duodeno de pacientes adultos con EoE.

7 – Conocer el efecto del tratamiento dietético eficaz sobre la reducción de la densidad de las células inflamatorias en el epitelio de pacientes adultos con EoE, así como los cambios en la expresión génica diferencial de los distintos TLRs, sus mediadores y efectores en la mucosa esofágica y duodenal.

8 – Proponer una hipótesis integrativa de la fisiopatología de la EoE, que incluya el papel potencial de los componentes de la respuesta inmune innata en el origen y mantenimiento de la respuesta inflamatoria característica de la EoE.

METODOLOGÍA Y RESULTADOS

Artículo 1: Incidence and prevalence of eosinophilic oesophagitis increase continiously in adults and children in Central Spain: A 12-year population-based study.

Dig Liver Dis. 2019 Jan;51(1):55-62
doi: 10.1016/j.dld.2018.07.016
***JCR Factor de Impacto: 3,287**;*
*Categoria: Gastroenterology & Hepatology 37/80 (**Q2**)*

Digestive and Liver Disease 51 (2019) 55–62

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

Incidence and prevalence of eosinophilic oesophagitis increase continiously in adults and children in Central Spain: A 12-year population-based study

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ARTICLE INFO

Article history:
Received 10 April 2018
Received in revised form 17 July 2018
Accepted 19 July 2018
Available online 31 July 2018

Keywords:
Eosinophilic esophagitis
Incidence
Prevalence
Spain

ABSTRACT

Objectives: To update population-based incidence, prevalence and trends for eosinophilic oesophagitis (EoE) in children and adults over the past decade.
Methods: All patients referred to our EoE unit and living in the study area up to December 2017 were prospectively registered. Endoscopy and pathology databases and clinical charts were manually reviewed. Diagnosis of EoE was confirmed upon symptoms of oesophageal dysfunction and eosinophilia >15 eos/HPF. Annual incidence rates and prevalence were estimated with confidence intervals (CI) of 95%.
Results: A total of 117 patients, including 19 children, were diagnosed with EoE in the 2006–2017 period. In 2017, the prevalence of EoE in children was 111.9 (95%CI, 67.4–174.6) cases/100,000 inhabitants and in adults 111.9 (95%CI, 90.8–136.5) also, and in both cases was significantly higher for male patients. The highest prevalences were observed in ages ranging between 20 and 24 and 35–39 years old. Mean incidence rates of the study period were 10.6 and 9.1 new cases/100,000 inhabitants/year in children and adults, respectively. Rise in the appearance of EoE during the study period exceeded that for endoscopic procedures. No seasonal variation was observed in the diagnosis of EoE.
Conclusion: The incidence and prevalence of EoE has increased sharply in central Spain, beyond previous estimations, with one out of every 893 inhabitants now being diagnosed.

1. Introduction

Eosinophilic oesophagitis (EoE) is an immune-mediated, oesophageal chronic disease, clinically characterized by symptoms of oesophageal dysfunction and histopathologically by the presence of oesophageal eosinophilia [1]. EoE constitutes a particular allergic condition triggered and maintained by food allergens [2], with a potential role for aeroallergen exposure in the genesis and exacerbations of EoE which is not supported by most of the current evidence [3,4].

First described in the early 1990s, and after years of being overlooked, the awareness of EoE has increased substantially in the last decade, to the point that it is currently the second cause of chronic oesophageal inflammation after gastro-oesophageal reflux disease (GORD) and the most common cause of dysphagia and food impaction among children and young adults [1]. A continuous increase in incidence rates and prevalence of EoE has been reported during recent years, which were summarized in 2016 in a systematic review with meta-analysis on population-based studies. The analysis gave a pooled incidence of 3.7 per 100,000 inhabitants/year and prevalence of EoE of 22.7 cases per 100,000 [5], but with a high inconsistency (I^2 99.9%) among the studies documented. EoE however still has a significant diagnostic delay [6,7], which does not seem to decrease despite the cumulative knowledge on the disease [8]. The initial doubts on whether the EoE epidemic could be fully explained by a growth in endoscopic examinations or improved detection and recognition of the disease by endoscopists and pathologists have been clarified by demonstrating a true increase in disease incidence that exceeds the expanding use of endoscopy [9–11]. Apart from a true increase in the cumulative incidence of the disease in multiple settings, the variations reported in the frequency of EoE in several studies has been as a

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https://doi.org/10.1016/j.dld.2018.07.016
1590-8658/

result of differences in the methodological approaches used (from population-based research to studies defining the frequency of EoE in series of endoscopies and databases of biopsies) and/or variations in the diagnostic criteria considered [12,13]. Differences in the threshold of eosinophil count that define EoE and in how patients with a response to proton pump inhibitors (PPIs) were characterized were the most relevant variations. With regard to this latter issue, guidelines for EoE published between 2007 and 2013 systematically recommended ruling out EoE, and providing a diagnosis of PPI-responsive oesophageal eosinophilia (PPI-REE) for those patients with clinical, endoscopic and histological features characteristic of EoE who achieved remission on PPI therapy [14–16]. Cumulative evidence later showed that EoE patients who did or did not respond to PPI therapy were genetically, molecularly, mechanistically, and phenotypically indistinguishable from each other, and radically different from those with conventional GORD [17–20]. Clinical and pathological features also remit after dietary or topic steroid therapy in PPI-REE patients [21,22], eliminating the therapeutic differences between both groups of patients. An international consensus report [23] and updated evidence-based guidelines [1] now consider PPI-REE as true EoE patients. Some previous research excluded the former, likely underestimating the burden of the disease.

Finally, all previous research evaluating the frequency of EoE exclusively analyzed paediatric or adult patients from different populations [5], which represents an additional limitation when evaluating the overall epidemiology of the disease in a given setting.

The aims of this study are (i) to assess the overall incidence and prevalence of EoE along the different ages in a central region of Spain for the period 2006–2017; and (ii) to analyze trends in epidemiological figures over time.

2. Methods

2.1. Study setting

This study was conducted in a health area located in the autonomous region of Castilla-La Mancha, in central Spain. It provides an update on previous research on EoE prevalence for the period up to 2011 [24]. The study area is predominantly rural, with an overall reference population of 104,737 inhabitants in 2017 with no relevant demographic changes in the study period (the variation coefficient between 2006 and 2017 was 3.6%). The area is covered by two neighbouring public hospitals belonging to the regional health service: Hospital General of Tomelloso and Hospital Virgen de Altagracia. Both hospitals offer universal coverage for specialized services, and their Departments of Gastroenterology and Paediatrics act as referral centres in the area since no additional private gastroenterology, endoscopy, paediatric, nor pathology clinics exist. The Hospital General de Tomelloso acts as a reference centre for EoE cases in the region, and centralizes all cases diagnosed at both centres. All patients included in the study or their parents gave their informed consent for endoscopic procedures to be undertaken and for inclusion in the EoE registry. The investigation was conducted according to the principles expressed in the Declaration of Helsinki and the registry supporting the study was approved by the Institutional Review Board at Hospital General La Mancha Centro.

Reference populations of the areas studied were obtained from official databases from the National Institute of Statistics for the same study period, according to which 87,753 (83.8%) adults older than 16 year-olds and 16,984 (16.2%) children, were living there. Gender percentages (%) distributions (M/F) in 2017 were 49.9/50.1 and 51.3/49.7 for children and adults, respectively.

2.2. Diagnostic criteria and case identification

The diagnosis of EoE was based on the presence of gastrointestinal symptoms suggestive of oesophageal dysfunction (e.g. dysphagia, food impaction, heartburn, reflux, chest pain, vomiting, epigastric/abdominal pain) and infiltration of oesophageal biopsies by 15 or more eosinophils per high-powered field (eos/HPF), in agreement with the criteria established in evidence-based clinical guidelines on EoE [1]. Other potential causes of oesophageal eosinophilia, including eosinophilic gastroenteritis, Crohn's disease, drug hypersensitivity, parasites, oesophageal caustications, hypereosinophilic syndrome, vasculitis, pemphigoid, connective tissue disorder, and graft-versus-host disease were ruled out based on medical records. Patients with EoE responding to an eight-week PPI therapy were also included.

All patients newly diagnosed with EoE at either of the two hospitals between 1 January 2006 and 31 December 2017 were prospectively included in the EoE case registry at our centre. Clinical records, endoscopic registries and histological databases were manually reanalyzed to ensure an EoE diagnosis. A patient was considered to be an adult if he or she was 16 years of age or older.

2.3. Data extraction

Demographic and allergologic data, age at diagnosis, type of symptoms, endoscopic features and peak eosinophil counts in baseline biopsies were recorded. Duration of symptoms before achieving a diagnosis was defined as "overall time of evolution"; time from first consultation with a physician (generally a primary care or general practitioner/paediatrician) to EoE diagnosis was defined as "diagnostic delay". Seasonal distribution of EoE diagnoses along the year was defined according to aerobiological information provided by the Spanish Society of Allergy and Clinical Immunology for the Ciudad Real province [25]. The pollen season in our region was defined from March to July, according to pollen count information.

2.4. Incidence and prevalence analyses

The annual incidence of EoE was calculated as the number of new patients identified for each year of study divided by the overall population in the study area of the corresponding year. Prevalence was estimated as the cumulative number of patients with EoE for each year divided by total population of our area of the corresponding year. Subgroup analyses by children and adults were performed. Confidence intervals (CI) of 95% were estimated for each value.

2.5. Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables as percentages. A comparison between children and adults were performed; t student test or U-Mann Whitney test were used for continuous variables and χ square test for categorical variables. A parametric correlation test (Pearson's r) was used for analysing the association between the overall number of cases diagnosed from EoE each year and the annual rate of upper endoscopy exam performed in the recruiting hospitals during the study period.

The binomial test was used to evaluate the deviations in distribution of observed EoE patients diagnosed within a time interval (season) to a theoretically expected distribution, assuming a probability of 0.25. Analyses and summaries were produced with the PASW statistical program, version 18.0 (SPSS, Inc, Chicago, Ill). A 0.05 level of significance was used throughout.

3. Results

3.1. Baseline characteristics

During the study, an overall of 234 patients attending our centres were diagnosed with EoE. 117 (50%) of these were living in our health area and constituted the study cohort (Fig. 1).

Baseline characteristics of the included patients are summarized in Table 1. The mean age ± SD at diagnosis was 29.8 ± 14 (range 5–82) years old. The percentage of males was 87.2% (male/female ratio: 6.8/1). Main symptoms leading to diagnosis in both children and adults were dysphagia (63.2% and 75.3%, respectively; p ns) and food impaction (47.4% and 77.3%, respectively; p = 0.008). By contrast, children presented more commonly with vomiting (36.8% vs. 6.2%; p = 0.001), and weight loss (21.1% vs. 3.1%; p = 0.013).

Median diagnostic delay was 6.2 ± 10.8 months, with no significant differences observed between children and adults (5.9 vs. 8.5 months, respectively; p = 0.185). Significant differences were observed however in overall time of evolution (12 vs. 36 months, respectively; p = 0.007) and length of symptoms before first consultation (4 vs. 37.2 month, respectively; p = 0.002), when children were compared to adults (Table 1).

3.2. Paediatric patients

3.2.1. Incidence

Nineteen children were diagnosed with EoE during the study period, including 15 boys and 4 girls. The first child diagnosed with EoE in our area was in 2008. Incidence subsequently increased, peaking at 28.4 new cases per 100,000 inhabitants in 2015. Apart for this, it remained stable at around 10 cases per 100,000 annually, except for the years 2009 and 2011, when no children were diagnosed at all. The mean annual incidence rate in children during the study period was 10.6 cases per 100,000 inhabitants, being higher in boys (16.1 cases per 100,000/year) than in girls (4.7 cases per 100,000/year) (Table 2).

3.2.2. Prevalence

Consequently, the prevalence of EoE in children rapidly increased in our area during the years covered by our research, up to a period cumulative prevalence of 105.1 cases (95%CI, 67.4–174.6) per 100,000 inhabitants, and peaking at 111.9 cases per 100,000 inhabitants in 2017. The prevalence was almost three times higher in boys than in girls, being respectively 172 (95% CI, 96.3–283.5) and 48.4 (95% CI, 13.2–123.9) cases per 100,000 inhabitants (Table 2).

3.3. Adult patients

3.3.1. Incidence

Ninety eight adults (including 87 men and 11 women) were diagnosed with EoE in our region, the first being in 2006. The average overall incidence was 9.1 new cases per 100,000/year, with significantly higher numbers of male (16 per 100,000 annually) than female cases (2.1 per 100,000/year) (Table 3).

3.3.2. Prevalence

The period cumulative prevalence from 2006 to 2017 was 107.7 cases diagnosed per 100,000 adult inhabitants, with a peak for prevalence adjusted by effective population in 2017 of 111.9 (95% CI, 90.8–136.5) cases per 100,000 inhabitants. For males, the period prevalence in 2017 was 199.7 (95% CI, 159.8–246.6) per 100,000 and in females, it was 25.2 (95% CI, 12.6–45.1) per 100,000 (Table 3).

3.4. Prevalence by aged groups

The prevalence and incidence values were similar in children and adults (Fig. 1). A peak of prevalence was observed in the group of patients 20–24 years old followed by that of 35–39 years old, with 300 and 264 cases/100,000 inhabitants, respectively (Fig. 2). Up to half of the cases affected people aged between 20 to 39 years old.

3.5. Seasonality and upper endoscopies

No seasonal variation at the moment of diagnosis of EoE was found in our research, with similar incidence rates throughout the year: 25.6% of patients were diagnosed each spring, autumn and winter, while the remaining 23.2% of patients were diagnosed in summer (p = ns). A similar number of patients were diagnosed during the pollen and no-pollen seasons (45.3% vs. 54.7%, p = 0.191) (Supplementary Table 1).

Finally, no relationship between the increasing onset of EoE cases and the number of upper endoscopies carried out in the hospitals attending the study area was found (Pearson's r = 0.05 and p = 0.884) (Fig. 3).

4. Discussion

This population-based study documents a dramatic increase in the epidemiology of EoE in our region over the 12 last years to the point that it has doubled from the previous estimations provided in 2011, when a prevalence of 44.6 cases per 100,000 inhabitants and a mean annual incidence of 6.37 new cases per 100,000 was estimated [24]. Our results confirm the escalating epidemiologic trends reported for EoE in multiple settings, [9,26–28] according to which the prevalence of the disease has been increasing continuously since studies published before 2008, to represent a 5-fold increase in only one decade [5].

We have now documented a mean annual incidence of 9.1 new cases per 100,000 inhabitants, exceeding the overall incidence rate of 7.2 that was provided by a recent meta-analysis summarizing studies carried out between 2008 and 2015 [5]. The prevalence of EoE among adult patients of 111.9 cases per 100,000 inhabitants parallels figures recently reported from mid-western Spain for the 2007–2016 period (81.73 patients per 100,000 inhabitants) [10] and constitutes the highest prevalence for EoE reported so far. The incidence rate documented here for EoE currently equals that reported for Crohn's disease in European Countries, including Spain, which ranges between 8.6 and 9.9 new cases per 100,000/year [29–32], while prevalence almost equals the figures recently provided for Spain of 137.17 per 100,000 inhabitants [29].

As for children, an average incidence rate of 10.6 new EoE cases per 100,000 inhabitants, provides evidence that there is an increase, which has grown progressively over time, in the appearance of new EoE cases for this age group also. Population studies carried out before 2008 showed an overall annual incidence for paediatric EoE of 3.3 cases per 100,000 inhabitants, which increased to 7.3 for studies published after this date [5]. It should be remembered, however, that the very first cases of EoE were reported less than 4 decades ago [33–35] and the disease was characterized as a distinct clinico-pathological disorder only in the early 1990s [36,37]. Since then, EoE has increased to the point that 1 out of 893 people are currently suffering from the disease in our region. Considering its chronic nature, the usual recurrence of symptoms and inflammation after treatment withdrawal [38] and the increasingly younger age of affected patients who will suffer from the disease for decades after being diagnosed, the burden of EoE for the national health services will be huge. Efforts to identify risk factors in order to

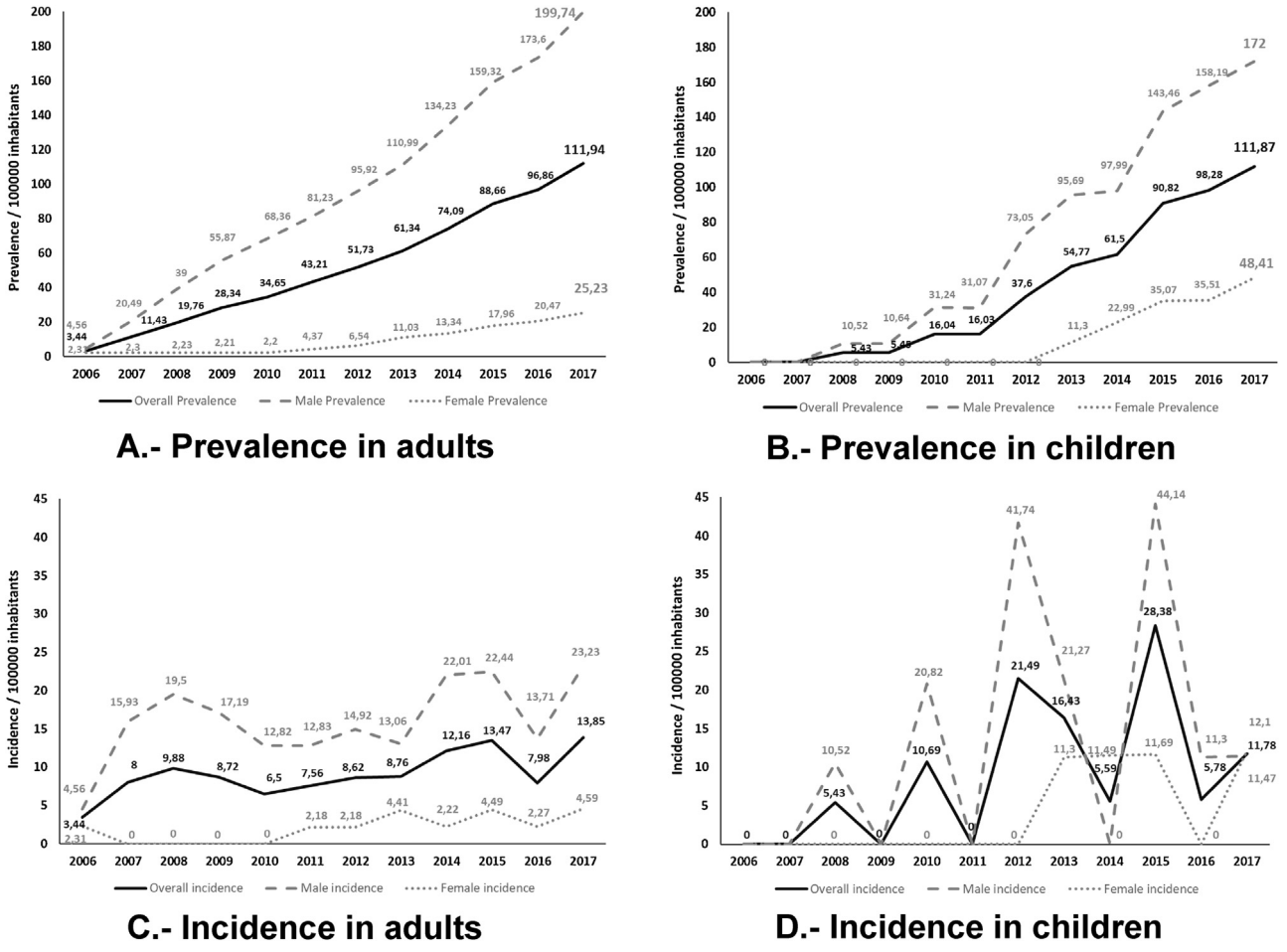

Fig. 1. Diagnostic incidence and cumulative prevalence of EoE per 100,000 inhabitants per year in adult and paediatric patients diagnosed in two hospitals in a central region of Spain during the period 2006–2017, according to evidence-based guidelines diagnostic criteria.

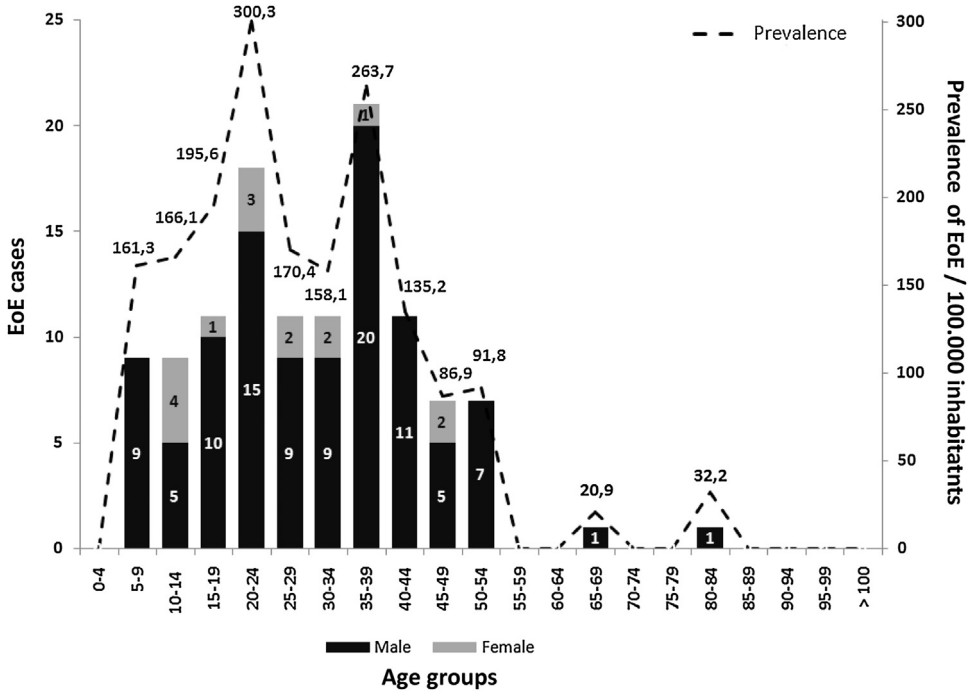

Fig. 2. New cases and prevalence rates of EoE, stratified by age group and gender, in patients diagnosed in a central region of Spain along a 12-year period.

Table 1
Baseline clinical and demographic characteristics of adult eosinophilic oesophagitis patients from two Spanish hospitals included in our study.

	Overall (n = 117)	Adults (n = 98)	Children (n = 19)	p
Mean age (SD; rank)	**29.8 (14; 5–82)**	33.8 (11.7; 16–82)	9.6 (2.8; 5–15)	**<0.001**
Male gender (%)	**102 (87.2%**)	87 (88.8%)	15 (78.9%)	0.263
Symptoms (%)				
Food impaction	**84 (72.4%)**	75 (77.3%)	9 (47.4%)	**0.008**
Dysphagia	**85 (73.3%)**	73 (75.3%)	12 (63.2%)	0.276
Abdominal pain	**31 (26.7%)**	25 (25.8%)	6 (31.6%)	0.601
Vomiting	**13 (11.2%)**	6 (6.2%)	7 (36.8%)	**0.001**
Heartburn	**27 (23.3%)**	21 (21.6%)	6 (31.6%)	0.378
Weight loss	**7 (6%)**	3 (3.1%)	4 (21.1%)	**0.013**
Reduced calibre (%)	**20 (17.1%)**	18 (18.8%)	2 (10.5%)	0.520
Mucosal appearance (%)				
Normal	**14 (12.3%)**	10 (10.5%)	4 (21.1%)	0.247
Longitudinal furrows	**85 (74.6%)**	72 (75.8%)	13 (68.4%)	0.566
Crepe-paper appearance	**24 (21.1%)**	20 (21.1%)	4 (21.1%)	>0.999
Rings	**56 (49.1%)**	55 (57.9%)	1 (5.3%)	**<0.001**
Exudates	**52 (45.6%)**	43 (45.3%)	8 (47.4%)	0.866
Atopic personal history (%)				
Rhinoconjunctivitis	**74 (63.8%)**	63 (64.9%)	11 (57.9%)	0.559
Asthma	**47 (40.5%)**	41 (42.3%)	6 (31.6%)	0.385
Food allergy	**29 (25%)**	20 (20.6%)	9 (47.4%)	**0.021**
Dermatitis	**7 (6%)**	5 (5.2%)	2 (10.5%)	0.322
Drug sensitivity	**10 (8.6%)**	9 (9.3%)	1 (5.3%)	>0.999
Atopic familiar history (%)				
Rhinoconjunctivitis	**23 (20%)**	20 (20.6%)	3 (16.7%)	>0.999
Bronchial asthma	**23 (20%)**	20 (20.6%)	3 (16.7%)	>0.999
Food allergy	**19 (16.5%)**	16 (16.5%)	3 (16.7%)	>0.999
Dermatitis	**2 (1.7%)**	2 (2.1%)	0	>0.999
Drug sensitivity	**5 (4.3%)**	4 (4.1%)	1 (5.6%)	>0.999
Mean peak eosinophils (SD; rank)	**58.1 (46.2; 15–300)**	59.5 (48; 15–300)	51.1 (36.1; 15–140)	0.467
Median overall time of evolution (IQR; rank), months	**36 (63; 0–360)**	36 (70.5; 0–360)	12 (27; 0–92)	**0.007**
Median Diagnosis Delay (IQR; rank), months	**6.2 (10,8; 0.2–128.7)**	5.9 (10.8; 0.2–128.7)	8.5 (18.2; 0.2–41.8)	0.185
Median length of symptoms before first consultation (IQR; rank), months	**31.8 (57.3; 0.6–343.1)**	37.2 (66.3; 1.4–343.1)	4 (20.1; 0.6–51.9)	**0.002**

Bold values denote statistically significant differences among children and adults.

Table 2
Annual incidence and cumulative prevalence for paediatric patients with EoE in two hospitals in central Spain between 2006 and 2017, broken down by gender. Incidence and prevalence are expressed in cases per 100,000 inhabitants.

Year	Overall	Male	Female	Overall population	Male population	Female population	Overall incidence	Male incidence	Female incidence	Overall prevalence	Male prevalence	Female prevalence
2008	1	1	0	18,419	9502	8917	**5.43**	10.52	0	**5.43**	10.52	0
2009	0	0	0	18,343	9402	8941	**0**	0	0	**5.45**	10.64	0
2010	2	2	0	18,703	9604	9099	**10.69**	20.82	0	**16.04**	31.24	0
2011	0	0	0	18,716	9655	9061	**0**	0	0	**16.03**	31.07	0
2012	4	4	0	18,616	9583	9033	**21.49**	41.74	0	**37.60**	73.05	0
2013	3	2	1	18,257	9405	8852	**16.43**	21.27	11.30	**54.77**	95.69	11.30
2014	1	0	1	17,885	9185	8700	**5.59**	0	11.49	**61.50**	97.99	22.99
2015	5	4	1	17,617	9062	8555	**28.38**	44.14	11.69	**90.82**	143.46	35.07
2016	1	1	0	17,298	8850	8448	**5.78**	11.30	0	**98.28**	158.19	35.51
2017	2	1	1	16,984	8721	8263	**11.78**	11.47	12.1	**111.87**	172	48.41
Overall	19	15	4	Mean 18084	9296.9	8786.9	Incidence **10.56**	16.13	4.66	Prevalence **49.78**	82.38	15.33

Bold values denote overall population-based annual incidence and cumulative prevalence figures.

implement preventive strategies to face EoE in future years are urgently needed therefore.

The rising prevalence of the disease cannot be attributed only to the accumulation of cases over time, but also to a continuous and ongoing increase in incidence rates. The reasons behind this increase have not been clarified but their identification is urgently needed. The true expansion in the prevalence and incidence of EoE in our area in respect to previous estimations in 2011, has not been adequately explained. It has been argued for example, that most of the previous population-based studies underestimated the magnitude of EoE by excluding patients with a response to PPIs [10]. All patients included in our research had EoE diagnosed by the current evidence-based criteria [1], according to which a response to PPI does not preclude a diagnosis of EoE, contrary to previous consensus guidelines [14,15]. However, and despite up to half of patients with EoE possibly responding to PPIs [39], most population-based studies carried out previously, both in the early literature [40–43] and in that published after the proposal of the so called PPI-REE in 2011 [9,26,27,44,45] did not exclude response to PPIs as a diagnostic requirement for EoE. As such, we can consider that previous literature assessing the prevalence and incidence rates for EoE did so by using equivalent criteria, as we used in the present research. An increasing generalization in the use of endoscopy for the diagnosis and management of gastroenterological disorders was also proposed as an explanation for an increasing frequency of EoE, together with a greater awareness by clinicians that now consider EoE within the differential diagnosis of oesophageal dysfunction symptoms [46,47]. However, recent studies have demonstrated that the increase in new EoE cases outpaces the use of endoscopy with biopsy [10,11]. Our research also documented that the expan-

Table 3
Annual incidence and cumulative prevalence of eosinophilic esophagitis in adult patients attended in two hospitals in central Spain between 2006 and 2017, broken down by gender. Incidence and prevalence are expressed in cases per 100 000 inhabitants.

Year	Overall	Male	Female	Overall population	Male population	Female population	Overall incidence	Male incidence	Female incidence	Overall Prevalence	Male Prevalence	Female Prevalence
2006	3	2	1	87274	43893	43381	**3.44**	4.56	2.31	**3.44**	4.56	2.31
2007	7	7	0	87477	43930	43547	**8**	15.93	0	**11.43**	20.49	2.30
2008	9	9	0	91071	46155	44916	**9.88**	19.50	0	**19.76**	39	2.23
2009	8	8	0	91744	46541	45204	**8.72**	17.19	0	**28.34**	55.87	2.21
2010	6	6	0	92340	46809	45531	**6.50**	12.82	0	**34.65**	68.36	2.20
2011	7	6	1	92564	46781	45783	**7.56**	12.83	2.18	**43.21**	81.23	4.37
2012	8	7	1	92781	46912	45869	**8.62**	14.92	2.18	**51.73**	95.92	6.54
2013	8	6	2	91294	45950	45344	**8.76**	13.06	4.41	**61.34**	110.99	11.03
2014	11	10	1	90436	45444	44992	**12.16**	22.01	2.22	**74.09**	134.23	13.34
2015	12	10	2	89102	44565	44537	**13.47**	22.44	4.49	**88.66**	159.32	17.96
2016	7	6	1	87753	43779	43974	**7.98**	13.71	2.27	**96.86**	173.60	20.47
2017	12	10	2	86652	43055	43597	**13.85**	23.23	4.59	**111.94**	199.74	25.23
Overall	98	87	11	Mean 90041	Mean 45318	44723	**9.08**	Mean Incidence 16.01	2.05	**52.12**	Mean Prevalence 95.78	9.18

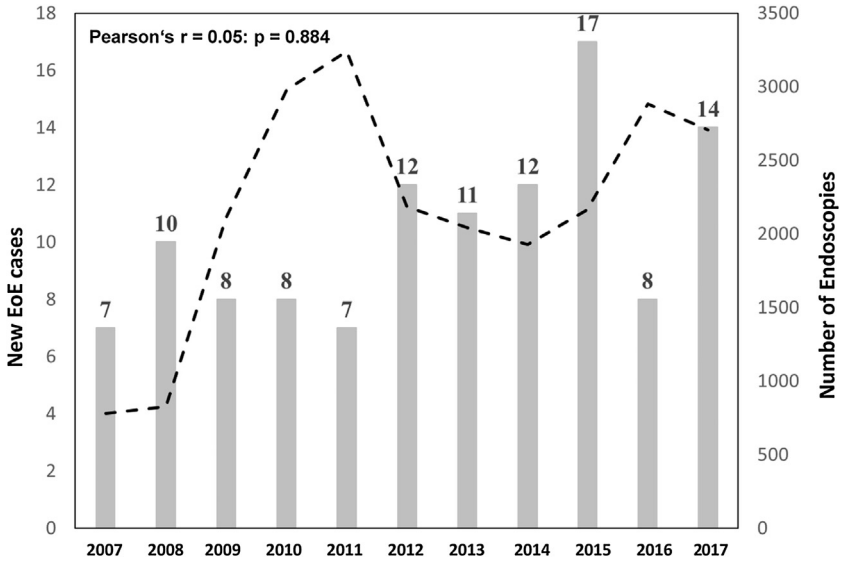

Fig. 3. Yearly overall new EoE cases diagnosed in children and adults in a central region of Spain between 2006 and 2017, compared to the annual rate of upper endoscopic exams performed in our hospitals during the same study period.

sion in the number of new EoE diagnoses during the study period exceeded that of upper endoscopic examations, thus providing additional evidence of a true expansion of the disease in our setting over the last decade.

Some of our results deserve additional comments. To begin with and in line with previous findings [10,27] the prevalence of EoE in our series steadily increased from childhood to adolescence, to peaks in the age groups 20–24 years old and 35–39 years old, which were the ages with the highest number of EoE diagnoses in our cohort. After 45 years old, the number of EoE cases sharply decreased, with only a scattering of patients being diagnosed at older ages. This finding confirms EoE as predominantly a disease of young adults, and invites speculation as to the moment that the as yet to be identified potential risk factors leading to EoE became active. At present, only early-life factors, including caesarean delivery, antibiotic or acid suppressant use in infancy and not having a pet in the home, have been related with an increased risk of suffering from EoE [48]. Considering the more common ages of disease diagnosis after early childhood, two conclusions can be drawn: (a) the latency period between exposure and disease diagnosis (but not onset) ranged between 20 and 40 years for the majority of patients; (b) the most pronounced effect of such factors started after the 1980s. Secondly, our results provided additional evidence on the lack of a seasonal predominance in the appearance of EoE [10], with a similar number of cases being diagnosed throughout the four seasons and with no significant effect of the pollen season on the number of new cases identified. In fact, a previous meta-analysis with meta-regression already demonstrated no seasonal distribution at the moment of diagnosis or clinical recrudescence of EoE [4]. It attributed the increased recognition of EoE during spring and summer to a greater opportunity for establishing a diagnosis in patients with mild, chronic oesophageal symptoms, instead of implicating outdoor antigens as potential EoE triggers. Finally, our mean diagnostic delay of only 6.2 ± 10.8 months from first consultation to definitive EoE diagnosis was significantly shorter than in previous research [6,8,10,49]. Overall time of evolution was also significantly shorter than that reported by other authors, which lasted around 5 years. The limited size of our area and easy access to specialized facilities, the fact of being a reference unit for EoE and the well established awareness of EoE among the staff of our recruiting area might have contributed to a greatly reduced diagnostic delay.

Our study has several strengths, such as the systematic and prospective identification of a large number of new EoE cases in patients of all ages. These patients consecutively attended a referral centre over a long period of time, for whom registered data

were cross-checked with endoscopy and pathology databases. The systematic inclusion of patients with EoE who responded to PPI therapy in accordance with current consensus [23] and evidence-based diagnostic criteria [1] should also be considered. The lack of alternative private clinics able to provide endoscopy services in our study area avoided the loss of potential EoE cases.

Some limitations should also be acknowledged, such as the fact that our data was obtained from patients who sought assistance because of the symptoms of oesophageal dysfunction, and were diagnosed after referral to the gastroenterology or paediatrics departments at our hospitals. In most of the previous research, diagnostic delay in EoE was considerable, significantly higher to that documented in ours [6,8,10,49], probably due to the fact that symptoms can fluctuate over time and are frequently unspecific. Thus, it can be assumed that only the most symptomatic patients would have been seen by primary care physicians and referred to hospital for additional studies. It is even likely that some young patients, with no alarming GORD-related symptoms and responding to empiric antisecretive therapy, were never referred for endoscopy. In this scenario, the finding of a pathological eosinophilic infiltration over the EoE diagnostic threshold described in Sweden during the Kalixandra study [50], would really reflect the actual magnitude of EoE. The study area is exclusively rural, with the local economy based mainly on agriculture, farming processing industries and community services. Therefore, caution should be taken before directly extrapolating our results to urban populations. Conflicting results have been shown regarding the differences in EoE frequency and living areas, with early research showing that EoE was spread homogeneously according to population distribution, with no urban–rural gradient [40]. An American epidemiological study later documented higher prevalence of EoE in urban areas compared with suburban and rural settings [51], and more recently population density has been strongly and inversely associated with oesophageal eosinophilia and EoE [52] suggesting that environmental exposures that are more prominent in rural areas may be relevant to the pathogenesis of EoE, a fact that we should also have considered.

In conclusion, the incidence and prevalence of EoE in our region, located in central Spain, has increased sharply throughout the last 12 years in patient of all ages. The present study reports the highest prevalence seen so far for paediatric and adult EoE and provides evidence of the markedly increasing trend in the frequency of EoE documented in several settings. In view of these results, efforts to investigate the causes of EoE and its increasing frequency in order to propose preventive strategies, are urgently needed.

Conflict of interest

None declared.

Guarantor of the article

Alfredo J. Lucendo.

Disclaimers

None.

Writing assistance

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.07.016.

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Artículo 2: Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis.

Clin Exp Allergy. 2016 Jan;46(1):78-91.
doi: 10.1111/cea.12504
***JCR Factor de Impacto: 5,587**;*
*Categoria: Allergy 26/15 (**Q1**) & Immunology 26/151 (**Q1**)*

doi: 10.1111/cea.12504 *Clinical & Experimental Allergy*, **46**, 78–91

ORIGINAL ARTICLE **Clinical Mechanisms in Allergic Disease**

Dietary treatment modulates mast cell phenotype, density, and activity in adult eosinophilic oesophagitis

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Clinical & Experimental Allergy

Summary

Background Mast cells (MCs) are abundant in the inflammatory infiltrate in eosinophilic oesophagitis (EoE), but decrease with disease remission. However, their phenotype, role in the pathophysiology of the disease, and modulation after effective dietary therapy are still unclear.

Objective To define the phenotype of oesophageal MCs, their modulation through dietary therapy, and their association with clinical manifestations of EoE.

Methods Oesophageal mucosal samples from 10 adult patients with EoE obtained before and after effective six-food elimination diet (SFED) therapy, as well as from 10 control subjects were analysed. Eosinophil and MC density were quantified. Gene expression of chemoattractants for eosinophils (CCL11, CCL24, and CCL26), MCs (SCF), and their receptors (CCR3 and SCFR, respectively) were assessed by means of qPCR. Gene and protein expression of specific MC proteases (CPA3, CMA, and TPSB2) were evaluated with qPCR and immunofluorescence. Clinical manifestations and atopic background were recorded.

Results MC density was significantly increased in EoE compared with controls, decreasing after dietary treatment (18.6 to 1.44 cells/hpf, respectively; $P < 0.001$). The MC_{TC} subtype predominated in the oesophageal mucosa (90%) in both patients with EoE and controls. Gene expression of MC-related proteases, eotaxins, and SCF were up-regulated in patients with EoE, but significantly decreased after therapy, regardless of atopic background. Epithelial peaks of MCs and eosinophils were significantly associated ($\rho = 0.80$) in EoE and correlated with the symptom score ($\rho = 0.78$). Gene expression of MC proteases and eotaxins also correlated with the symptom score ($P < 0.05$).

Conclusions and Clinical Relevance MC and its proteases seem to play a relevant role in the pathophysiology and symptoms of EoE, which can be reversed after effective dietary treatment.

Keywords carboxypeptidase A3, CCL24, CCL26, CCR3, chemokines CCL21, chymase, dietary treatment, Eosinophilic oesophagitis, mast cells, SCF, SCFR, six-food elimination diet, tryptase

Submitted 23 July 2014; revised 11 November 2014; accepted 14 November 2014

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Cite this as: Á. Arias, A. J. Lucendo, P. Martínez-Fernández, A. M. González-Castro, M. Fortea, J. González-Cervera, J. L. Yagüe-Compadre, T. Mota-Huertas, M. Vicario, *Clinical & Experimental Allergy*, 2016 (46) 78–91.

Introduction

Eosinophilic oesophagitis (EoE) is a chronic food-triggered, immune-mediated disease of the oesophagus. Clinically, EoE is characterized by symptoms of oesophageal dysfunction, while histologically, it is marked by an inflammatory infiltrate with large numbers of both intraepithelial eosinophils and mast cells in the oesophageal epithelium [1]. In the past few years, EoE has rapidly risen in both incidence and prevalence [2–4] so

that it is now the most likely cause of dysphagia among young patients.

A role for mast cells in the pathogenesis of the disease has been proposed [5–8] after studies demonstrated both their activation [8] and increased density in the oesophageal mucosa of experimental [9, 10] and human EoE in adults [11–14] and children [8, 15–19]. These increases were significant compared with healthy controls as well as with patients with gastro-oesophageal reflux disease (GERD); in fact, mast cell density has been proposed as a marker to distinguish GERD from EoE [15, 20].

The potential role played by mast cells in EoE is supported by several pieces of evidence, most of it indirect. For example, the density of mast cells correlates with eosinophilic infiltration within the oesophageal epithelium [21], with a reduction in both cell types after treatment with topical steroids [22–24] or anti-interleukin-5 [25] and in association with clinical remission [12, 24, 26]. The expression of specific mast cell-mediators has also been shown to be up-regulated in several reports [8, 16, 18], with mast cell-derived TGF-β1 contributing to oesophageal dysmotility in both human [18] and experimental (murine) EoE [9] through the induction of smooth muscle hypertrophy and hyperplasia. Previous research supports the role of these cells in local IgE-mediated reactions against certain allergens, as IgE production and IgE^+ mast cells are present in the oesophageal epithelium of these patients [13, 19]. However, their contribution to the aetiopathogenesis of EoE remains unclear.

Mast cells are mesenchymal bone marrow-derived myeloid cells that are widely distributed in vascular connective tissue as a part of the innate immunity elements against parasites and bacteria. Human mast cells are classified into two types depending on their granule content [11, 27]: MC_T (mast cells with tryptase) and MC_{TC} (mast cells with tryptase and chymase). Typically, MC_T are located in the mucosal tissue while MC_{TC} are found mainly in connective tissues, but they can also be found in the submucosa and, rarely, in the muscularis propria of the digestive tract [28–30]. This phenotypic diversity is not only a descriptor of tissue location [31], but also of the regulation of cytokine gene expression and, as such, is associated with functional differences [32].

In recent years, dietary therapies have emerged as a drug-free treatment alternative for inducing and maintaining disease remission in both paediatric and adult patients with EoE [33]. According to a recent systematic review [34], an empiric six-food elimination diet (SFED) is currently the best dietary approach for inducing histological remission of EoE. The anti-inflammatory properties of SFED are exerted by removing antigenic luminal stimuli from the diet of sensitized patients [35–38], allowing the recovery of oesophageal tissues without inducing apoptosis in inflammatory cells or modifying signalling pathways, which commonly occurs when steroids, immunomodulators, or biological therapies are used. Despite mast cells being the main effector cells in IgE-associated responses and playing a central role in allergic responses [39], to date, the ability of dietary therapies to reduce mast cell density and/or activity has not been fully elucidated.

The aims of this study were to analyse the phenotype of oesophageal mast cells and the effect of an SFED on the eosinophil and mast cell infiltrate in EoE. The contribution of mast cell activity to clinical remission will also be studied to gain further insight into the aetiopathogenic mechanisms of this disease.

Material and methods

Study design

A controlled, quasi-experimental design was used. Patients with EoE and control subjects were recruited, and clinical symptoms were recorded. Oesophageal biopsies were obtained from each participant at baseline and, in patients with EoE, after 6 weeks of an empiric SFED. Biological assessment of tissue samples and clinical evolution were analysed to evaluate the response to dietary treatment.

Participants and clinical assessment

Adult patients with EoE who were naïve to topical or systemic steroid therapy for EoE were prospectively recruited from October 2011 through March 2012. Diagnosis for EoE was based on widely accepted criteria [1] which included (i) infiltration of oesophageal epithelium by 15 or more eosinophil leucocytes per high-powered field (hpf); (ii) absence of eosinophilic infiltration in biopsy specimens from gastric and duodenal mucosa; (iii) ruling out of proton pump inhibitor-responsive oesophageal eosinophilia as defined by the persistence of eosinophilic infiltration after an 8-week course of omeprazole (20 mg/twice a day); and (iv) ruling out drug intake, parasites, oesophageal causticications, haematologic neoplasms, or other events in the patient's medical history as possible causes of oesophageal eosinophilia.

Gender-matched control samples were obtained endoscopically from individuals who had been consecutively referred to undergo endoscopy under sedation during the study period due to symptoms of dyspepsia or a suspected gastroduodenal ulcer. All selected control subjects exhibited a normal endoscopic appearance of the oesophagus; hiatal hernia, incompetent cardias, and oesophageal peptic lesions were excluded, and the

analyses of oesophageal mucosal biopsies were also reported as normal. Wherever possible, clinical histories of all participants were used to assess family and/or personal background of atopy (Table 1; see also Table S1).

Oesophageal symptoms were assessed structurally by means of a score validated for achalasia [40], but previously used in adult EoE [37, 41]. The duration and intensity of the dysphagia events along with the frequency and intensity of heartburn and regurgitation were recorded both at the beginning of the study and after dietary treatment.

Endoscopy and biopsy sampling procedure

All endoscopic exams were carried out under conscious sedation by a board-certified gastroenterologist (AJL); they were performed with a flexible 9-mm-calibre Pentax EG-2770K gastroscope (Pentax of America, Inc, Montvale, NJ, USA) with a 2.8-mm work channel. The calibre and appearance of the oesophageal wall were recorded for all participants during the endoscopic procedure. Biopsies were taken with the aid of a standard needle biopsy forceps (Endo Jaw FB-220U, Olympus Medical Systems, Tokyo, Japan) from both the upper and lower oesophageal thirds; a minimum of five specimens were obtained from each location. These were then fixed in 4% formalin and routinely processed for histopathological analysis. Three additional endoscopic samples from the middle oesophageal third of all study subjects were collected during the same endoscopic procedure and preserved in an RNA stabilization solution (RNAlater; Ambion, Inc, Austin, TX, USA) at −80 °C until being processed for gene expression studies. No specific complications were observed in any patient after the biopsy procedure.

Treatment and follow-up period

All patients diagnosed with EoE were asked to follow an SFED for a 6-week period, avoiding the consumption of six-food groups reported to cause food allergies, namely cereals, milk and dairy products, eggs, fish/seafood, soya/legumes, and nuts [37]. The patients were given an amino acid-based formula adapted to oral consumption (Neocate Advance, 100 g sachets, banana & vanilla flavours, SHS International, Liverpool, UK) in order to supplement their diets. Written information about which foods should be avoided and which allowed, along with instructions to read food labels carefully, were provided to patients by board-certified gastroenterologists in our department. A telephone number and e-mail address were also provided to patients in case of further doubts regarding the SFED. Only oesophageal samples from patients who showed diet-induced remission of EoE were considered for comparative analysis.

Histological study

Oesophageal mucosal samples were fixed in formalin, embedded in paraffin, and routinely processed for haematoxylin and eosin staining. The histological analysis was performed by an experienced pathologist (JLY-C) blinded to the experimental groups. The peak number of eosinophils was counted in the most densely inflamed areas with the aid of Nikon Eclipse 50i (Nikon Corp, Tokyo, Japan) light microscopy in 3 high-power

Table 1. Clinical characteristics of patients with EoE included in the study

Patients	Age (years)	Sex	Time of evolution (months)	Symptoms	Endoscopy		Family background of atopy	Personal background of atopy	Identified trigger food
					Calibre	Mucosal appearance			
1	25	M	12	FI, Dy	N	LF, Rg	No	No	F&S &Ri
2	18	M	60	FI, WL	N	LF, C	Sister: D	AR	Le, Nu&Ri
3	38	M	4	Dy, AP	R	WP, Rg	No	BA, AR	Mi, Eg, Ri, F&S, Le & So
4	36	M	36	FI	N	LF, WP, Rg	Brother: FS	BA, AR	Mi, Ri, Nu& So
5	38	F	60	FI, Dy	N	LF, WP	Sister: AR	BA, AR	Leg&Nu
6	18	M	24	AP, V	N	LF, Rg	No	AR, FS	Mi, F&S, Le, Nu&Ri
7	51	F	24	FI, Dy	N	LF, WP, Rg	No	No	Mi & Le
8	34	M	48	FI, Dy, Ht	R	LF, WP, C, Rg	Father: BA; Brother: AR	No	Mi
9	38	M	120	FI, Dy	N	Normal	No	BA, AR, FS	Ri
10	35	M	120	Dy, AP	N	Rg, C	Brother: DS	BA, AR, FS	Mi, F&S &Ri

Sex: M, male; F, female. Symptoms: FI, food impaction, Dy, dysphagia, AP, abdominal pain, V, vomiting, Ht, heartburn, WL, weight loss. Endoscopy: N, normal; R, reduced; Rg, rings; LF, longitudinal furrows; C, crêpe-paper appearance; WP, white plaques. Atopy: BA, bronchial asthma; AR, allergic rhinitis; FS, food sensitivity; D, dermatitis; DS, drug sensitivity. Food triggers: Mi: milk; Ri: rice; F&S: fish & seafood; Le: legumes; Nu: nuts; Wh: wheat; Co: corn; Eg: eggs; So: soya.

fields (0.212 mm^2). Peak eosinophil count per hpf was calculated in the epithelial strata by averaging the eosinophil counts.

Immunofluorescence

Formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm. Cuts were first deparaffinized and rehydrated following standard procedures and then permeabilized with 0.1% Triton X-100 in PBS for 10 min. After treatment with blocking solution (DakoDiagnósticos, Barcelona, Spain) for 60 min at room temperature, samples were simultaneously incubated overnight at 4 °C either with the primary antibodies antitryptase (TPSB2, Dako) and antichymase (CMA, Abcam, Barcelona, Spain) or with antitryptase and anticarboxypeptidase (CPA Abcam). Samples then underwent a subsequent 30-min incubation at room temperature with the secondary antibodies Alexa Fluor 594 goat anti-rabbit IgG and Alexa Fluor 488 goat anti-mouse IgG (Life Technologies, Madrid, Spain). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The negative control slides were made in the same fashion except no primary antibodies were added. Fading was controlled using the Prolong antifade mounting medium (Molecular Probes). Positive cells in the epithelium, the papillae, and the lamina propria were counted with the aid of a fluorescence microscope (BX61, Olympus, Barcelona, Spain) at high magnification (400×) in 10–12 non-overlapping fields. Results are expressed as the number of positive cells/hpf in each anatomical location, as well as the percentage of CMA^+ or CPA^+ cells with respect to the $TPSB2^+$population.

Analysis of RNA expression

Total RNA was isolated with the Mir*Vana*™ miRNA Isolation Kit (Ambion), following the manufacturer's instructions. Gene expression for the chemotactic factors for eosinophils (CCL11, CCL24, and CCL26), mast cells (SCF and TGF-β), and their receptors (CCR3 and SCFR, respectively), along with mast cell-specific proteases (CPA3, CMA, and TPSB2) were evaluated in all samples. Each assay and its assay ID number are available at Applied Biosystems (Madrid, Spain) (see Table S2). Simultaneous real-time PCRs were performed with TaqMan Low-Density Arrays (Applied Biosystems) preconfigured in a 384-well format and spotted on a microfluidic card. Each TaqMan Gene Expression Assay consists of a forward and reverse primer at a final concentration of 900 nM and a TaqMan MGB probe (6-FAM dye-labelled; Applied Biosystems), with a final concentration of 250 nM. The assays are gene specific and have been designed to span an exon–exon junction. Thermal cycling conditions were 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min in an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). This procedure was replicated twice for each gene and each sample, with water as a negative control.

Relative changes in mRNA expression were calculated with the cycle threshold (Ct) method [42] with the aid of Sequence Detection System 2.1 software (Applied Biosystems). Expression levels of target genes were normalized to 18S, GAPDH, PGK1, GUSB, and β-actin expression.

The amount of mRNA for each gene was calculated in each sample using the Ct value. Relative gene expression was calculated as follows: $2\Delta\Delta Ct$, where $\Delta\Delta Ct = \Delta Ct$target gene $- \Delta ct$ control genes. The fold change for the treatment was defined as the relative expression compared with the corresponding control and was calculated as follows: $2\Delta\Delta Ct$, where $\Delta\Delta Ct = \Delta Ct$patient $- \Delta Ct$healthy.

Statistical analysis

We calculated the optimal sample size based on our previous results [37], from which we observed that patients with EoE had a mean eosinophil count of 47.9 (25.6) eos/hpf and that after dietary treatment, the number of eosinophils decreased significantly to 3.5 (3.9) eos/hpf. Drawing on these results and aiming for a power of 90%, five individuals would be needed to observe these differences. In the end, 10 patients were selected to detect possible differences in both mast cells and gene expression.

Means and standard deviations were reported for continuous variables and are expressed as 'mean (standard deviation)' throughout the text. Proportions were reported for categorical data. Results are expressed as a median with an interquartile rank (IQR) for scoring clinical symptoms. Comparisons between groups (control subjects and patients with EoE) were performed with nonparametric tests: the Mann–Whitney *U*-test for quantitative variables and the Fisher's exact test for nominal variables. For comparison before and after SFED treatment, the nonparametric-paired Wilcoxon signed-rank test was used. Nonparametric correlations (Spearman's rho) were used for relationships between eosinophils, mast cells, gene expression, and clinical symptoms. A 0.05 level of significance was used throughout. Statistical analyses were performed with the aid of PASW 18.0 statistical analysis software (SPSS Inc, Chicago, Ill).

Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board of our hospital. Informed consent was obtained from all patients prior to all endoscopic exams.

Results

Study population

A total of 10 patients with EoE (eight men) and 10 gender-matched control subjects were included in the analysis. The groups had a mean age of 33.1 (10.1) and 53 (19.9) years, respectively. Individual clinical characteristics of the experimental subjects are given in Table 1 and Table S2. Mean duration of symptoms in patients with EoE exceeded 4 years (50.8 ± 40.9 months), with dysphagia and food impaction being the most common, exhibited by 70% of patients. No difference in clinical manifestations was observed between atopic and non-atopic subjects (Table 2).

Eosinophils and mast cell density, chemoattractants, and the effects of dietary treatment

In the EoE group, peak intraepithelial eosinophil density was 56.8 (29.9) cells/hpf, which decreased to 3 (4.2) cells/hpf after SFED-based treatment ($P < 0.001$). No intraepithelial oesophageal eosinophils were detected in any of the controls. Peak counts for intraepithelial mast cells in patients with EoE were 18.6 (15.2) cells/hpf, much higher than for the control group, which had a peak count of 0.5 (0.6) cells/hpf ($P < 0.001$). As before, after SFED, mast cell density decreased to 1.44 (1.7) cells/hpf ($P < 0.001$) (Figs 1 and 2). No differences between atopic and non-atopic patients with EoE were detected in eosinophil [55 (30.4) and 61 (34.8) cells/hpf, respectively] or mast cell counts [20 (18.1) and 15.3 (5.2) cells/hpf, respectively].

Active eosinophil recruitment was demonstrated by identifying overexpression of all the eotaxins in the EoE group in comparison with the controls: CCL11 (8.5-fold increase), CCL24 (12.2-fold increase), and CCL26 (51.1-fold increase, $P < 0.05$ for all), which is in good agreement with previous studies [21, 43]. Dietary treatment significantly decreased eosinophil infiltration and all eotaxin expression to control group values

Table 2. Clinical characteristics and gene expression levels of atopic and non-atopic patients with EoE

		Atopic *vs.* Non-atopic	*P*
Time of evolution (months)		60.6 (45.1) *vs.* 28 (18.3)	0.250†
Symptom Score		9 (5.9) *vs.* 6 (2)	0.723†
Symptoms	Dysphagia	57.1% *vs.* 100%	0.475*
	Food impaction	57.1% *vs.* 100%	0.475*
	Abdominal pain	42.9% *vs.* 0%	0.475*
	Heartburn	0% *vs.* 33.3%	0.300*
	Vomiting	14.3% *vs.* 0%	> 0.999*
	Weight loss	14.3% *vs.* 0%	> 0.999*
Endoscopy Findings	Reduced calibre	14.3% *vs.* 33.3%	> 0.999*
	Normal mucosa	14.3% *vs.* 0%	> 0.999*
	Longitudinal furrows	57.1% *vs.* 100%	0.475*
	Rings	71.4% *vs.* 66.7%	> 0.999*
	Crêpe-paper appearance	28.6% *vs.* 33.3%	> 0.999*
	White plaques	42.9% *vs.* 66.7%	> 0.999*
Peak eosinophil count		55 (30.4) *vs.*61 (34.8)	0.908†
CCL11 gene expression		0.41 (0.94) *vs.* 0.15 (0.14)	0.425†
CCL24 gene expression		1.5 (2.1) *vs.* 0.91 (1)	0.732†
CCL26 gene expression		167 (165.1) *vs.* 275 (327.5)	0.305†
CCR3 gene expression		0.01 (0.01) *vs.* 0.08 (0.09)	0.305†
Peak mast cell count		17.4 (15.9) *vs.* 15.2 (10.5)	0.909†
TGF-beta gene expression		1.1 (0.3) *vs.* 0.89 (0.2)	0.305†
SCF gene expression		11.7 (10) *vs.* 13.6 (12.5)	0.909†
SCFR gene expression		9.3 (6.6) *vs.* 5.8 (4.4)	0.210†
CPA3 gene expression		18.6 (17.3) *vs.* 25.3 (65.1)	0.732†
CMA gene expression		2.1 (2) *vs.* 5.9 (6.5)	0.456†
TPSB2 gene expression		2.4 (3.9) *vs.* 4.4 (3.9)	0.909†

*Chi-square test. †Mann–Whitney *U*-test.

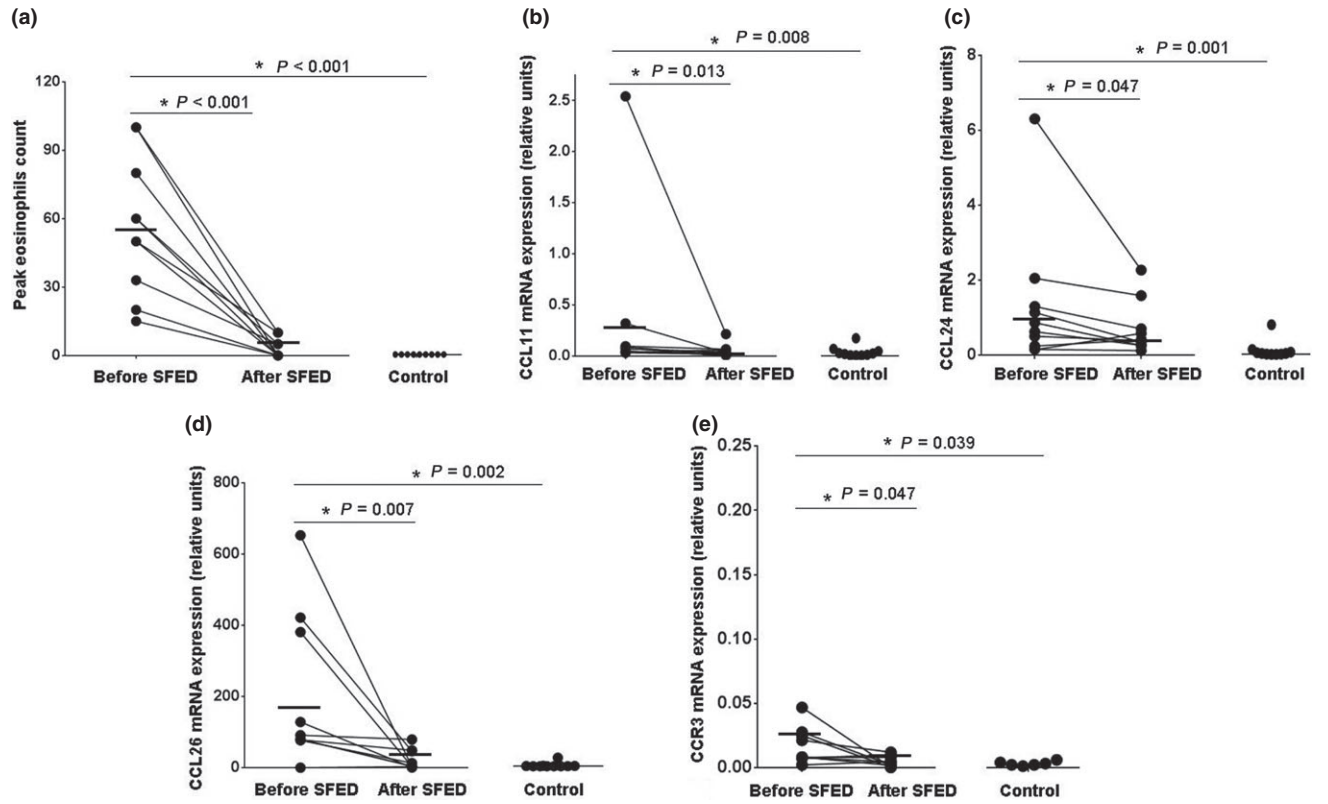

Fig. 1. Eosinophil density and expression of eosinophil chemoattractant molecules. (a) density of intraepithelial eosinophils in patients with EoE before and after effective treatment with a six-food elimination diet (SFED) and in control subjects. Gene expression of eosinophil–chemotactic chemokines eotaxin-1/CCL-11 (b); eotaxin-2/CCL24 (c); and eotaxin-3/CCL-26 (d) in oesophageal mucosal samples from patients with EoE, at baseline and after SFED-induced disease remission, compared with control samples. (e) changes in gene expression of eotaxin receptor CCR-3 in the same samples, at baseline and after an effective SFED. Individual changes in cytokine gene expression are provided. Horizontal bars represent means. *Statistically significant differences ($P < 0.05$) before and after treatment in patients with EoE.

($P < 0.05$). Moreover, the expression of CCR3, the common receptor for eotaxins, was also up-regulated (3.7-fold increase) in patients with EoE, decreasing to control levels after SFED-based treatment (Fig. 1).

In mast cells, chemotaxis was identified through an increase of mRNA in SCF and its receptor (SCFR). In patients with EoE, these values went up 5.6-fold and 3.7-fold, respectively ($P < 0.05$), in comparison with the control group. SFED-based treatment restored SCF gene expression to control values and also reduced SCFR, although not in a statistically significant manner (Fig. 2).

Mast cell phenotype and density in EoE and the effects of dietary treatment

In the control group, 100% of mast cells displayed the MCTC phenotype, although with low density, and 89.3% (±15.6) also contained CPA. In patients with EoE, the proportion of MC_{TC} cells decreased from 100% to 90.2% (±18.8) in the epithelium ($P = 0.020$), a reduction that was reversed after dietary treatment. No significant changes in the mast cell phenotype within the vascular papillae or the lamina propria were observed (data not shown). The number of CPA^+TPSB2^+ cells/hpf in the epithelium and the vascular papillae was higher in patients with EoE than in the controls. Dietary treatment reversed this increase in all tissues studied (Fig. 3). The density of CMA^+TPSB2^+ cells/hpf in the epithelium of active EoE was also reduced to control values after dietary treatment (Fig. 4).

Mast cell activation and modulation through dietary treatment

Mast cell activity was assessed by quantifying the gene expression of specific mast cell proteases. In EoE samples, all molecules were overexpressed in comparison with control samples: CMA (3.2-fold increase), CPA3 (3.2-fold increase), and TPSB2 (1.7-fold increase, $P < 0.05$ for all); all were reduced to control values ($P < 0.05$) after dietary treatment (Fig. 5). Moreover, no differences in mast cell counts or expression of mast cell-related genes were observed between atopic and non-atopic patients with EoE (Table 2).

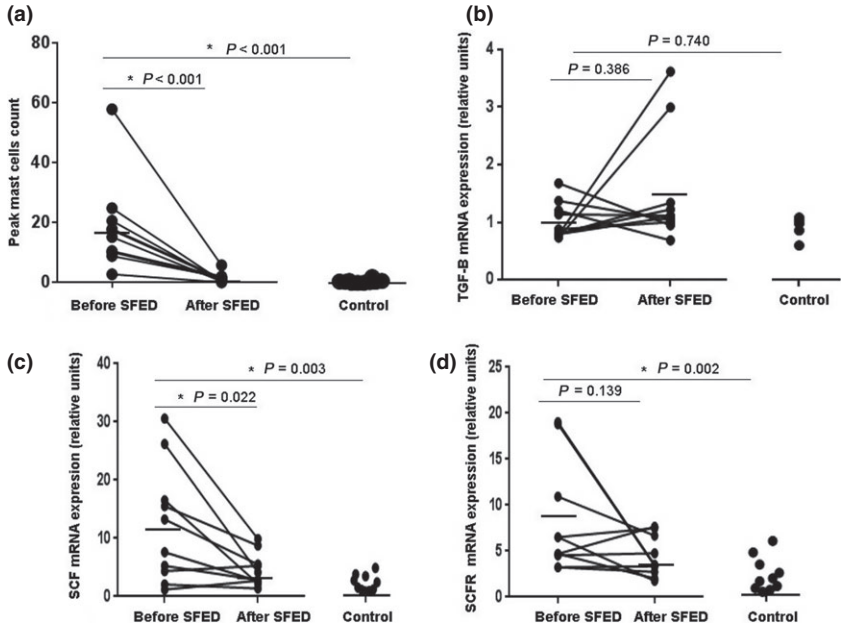

Fig. 2. Mast cell density and expression of mast cell chemoattractant molecules. (a) density of intraepithelial mast cells in patients with EoE before and after six-food elimination diet (SFED)-induced remission and in control subjects. (b) changes in mast cell-derived TGF-β gene expression in the same samples. (c) Gene expression of mast cell chemoattractant stem cell factor (SCF) and its receptor SCFR (d) in oesophageal mucosal samples from patients with EoE, at baseline and after SFED-induced disease remission, and in control samples. Individual changes in cytokine gene expression are provided. Horizontal bars represent means. *Statistically significant differences ($P < 0.05$) before and after treatment in patients with EoE.

Modulation of clinical symptoms through dietary treatment

EoE-associated symptoms were significantly reduced in every patient with EoE after dietary treatment (Fig. 6). Dysphagia (any intensity) was completely resolved in over 70% cases, while food impaction disappeared in 85% of patients. No significant differences in symptom scores in relation to the age or sex of the patients was observed nor did disease duration correlate with the degree of symptom score improvement (data not shown).

Relationship between eosinophils, mast cells, gene expression, and clinical symptoms

The number of eosinophils was significantly correlated to the number of mast cells in EoE oesophageal samples (r_s=0.808; $P < 0.001$). The density of both eosinophils and mast cells was strongly associated with the symptom score ($r_s = 0.895$ and $r_s = 0.782$; $P < 0.001$, respectively); likewise, cellular infiltration was also associated with gene expression of major chemotactic factors, including CCL26 ($r_s = 0.706$; $P = 0.001$ with eosinophils), CCL11 ($r_s = 0.452$; $P = 0.045$ with eosinophils), and SCF ($r_s = 0.39$; $P = 0.085$ with mast cells). These correlations were independent of atopic background.

There was also a significant association between the number and activation of mast cells in EoE, as demonstrated by the correlation between mast cell peak and gene expression of CPA3 ($r_s = 0.54$; $P < 0.05$), CMA ($r_s = 0.49$; $P < 0.05$), and TPSB2 ($r_s = 0.49$; $P < 0.05$) proteases. Moreover, mast cell protease expression was associated with oesophageal symptom score (Table 3). There was no association between the number of foods triggering EoE and the number of eosinophils ($P = 0.840$) or mast cells ($P = 0.832$) (Table 3).

Discussion

Our results demonstrate the effectiveness of dietary treatment in adult EoE, both in reducing mast cell density and activation, and in disease remission, providing proof of the major role these cells play in the pathophysiology of the disease. Moreover, we found that mast cell and eosinophil infiltration in the oesophageal epithelium were directly associated and significantly correlated with clinical symptoms in adult patients with EoE. Additionally, significant relationships between symptoms and the expression of major mast cell proteases were demonstrated as well as with chemoattractant stimuli for both cell types. Finally, to the best of our knowledge, this is the first time that researchers have determined that the mast cell population within the

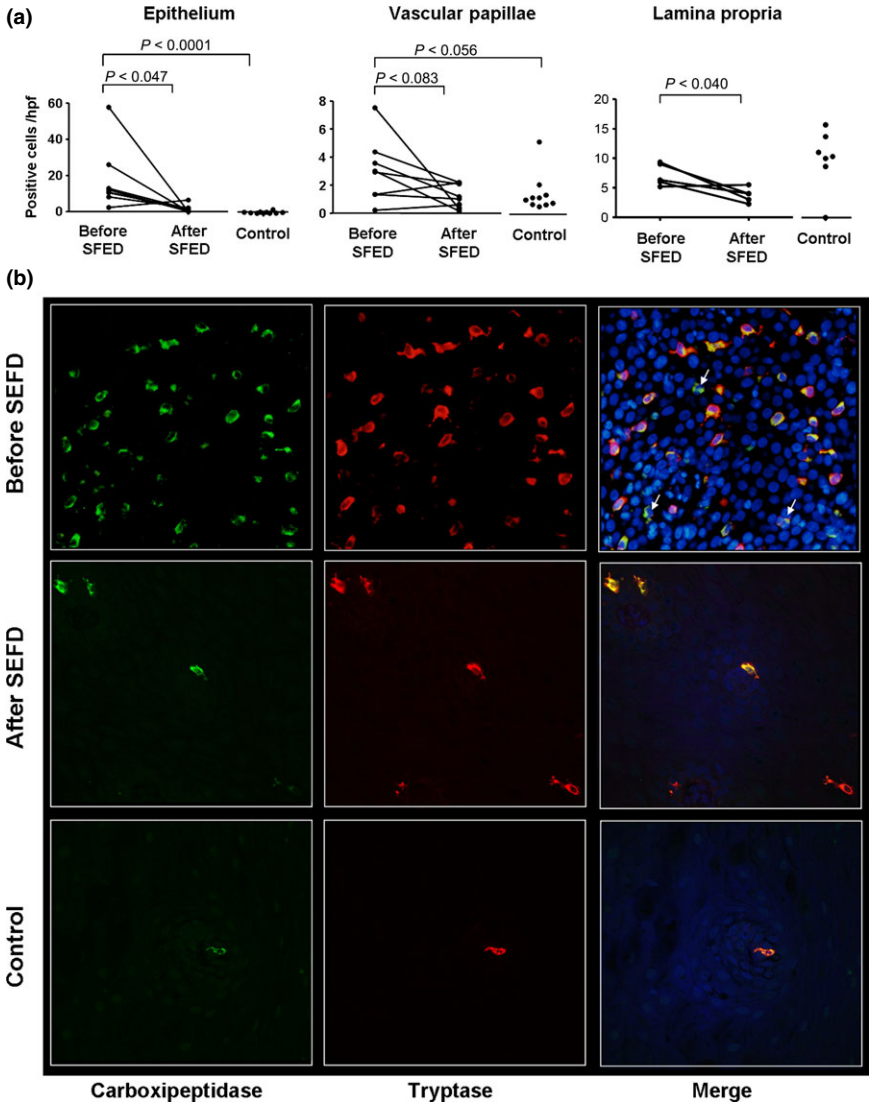

Fig. 3. Histological evaluation of mast cells in the oesophageal mucosa. (a) Individual cell counts per hpf of carboxypeptidase-positive cells in the epithelium, vascular papillae, and lamina propria of patients with EoE before and after dietary treatment, and in the control group. (b) Representative images of the double immunofluorescence for carboxypeptidase and tryptase staining in the three experimental groups. carboxypeptidase-positive mast cells infiltrate the epithelium and the vascular papillae in EoE. Dietary treatment reduced cell density and positive cells were then mainly detected in the vascular papillae. Eosinophils are identified within the epithelium, based on the nuclear morphology (white arrows). Note: SFED: six-food elimination diet.

oesophageal epithelium predominantly consists of MC_{TC} cells, both under normal conditions and in EoE. These cells are also predominant in the skin, nasal mucosa, and intestinal submucosa, but not in the small intestinal mucosa [44]. MC_{TC} cells do not specifically respond to mast cell-stabilizer drugs such as sodium cromoglycate in the same way as MC_T cells, which are predominant in the bronchial mucosa and alveolar wall, a finding which explains the documented lack of efficacy of these drugs in treating EoE [1, 45].

Antigen cross-linking of IgE antibodies on the mast cell surface is the most extensively studied mechanism for the activation and degranulation of these cells. This leads to the rapid release of autacoid mediators and the sustained synthesis and release of cytokines, chemokines, and growth factors [46], which can characteristically lead to anaphylaxis. However, immediate systemic reactions to the foods responsible for EoE are not described in these patients, despite the fact that local IgE production has been demonstrated in the oesophageal mucosa of patients with EoE regardless of their atopic background [19]. Moreover, IgE-bearing mast cells are present in the oesophageal epithelium of patients with EoE exhibiting a personal atopic history [6, 47]. It is worth

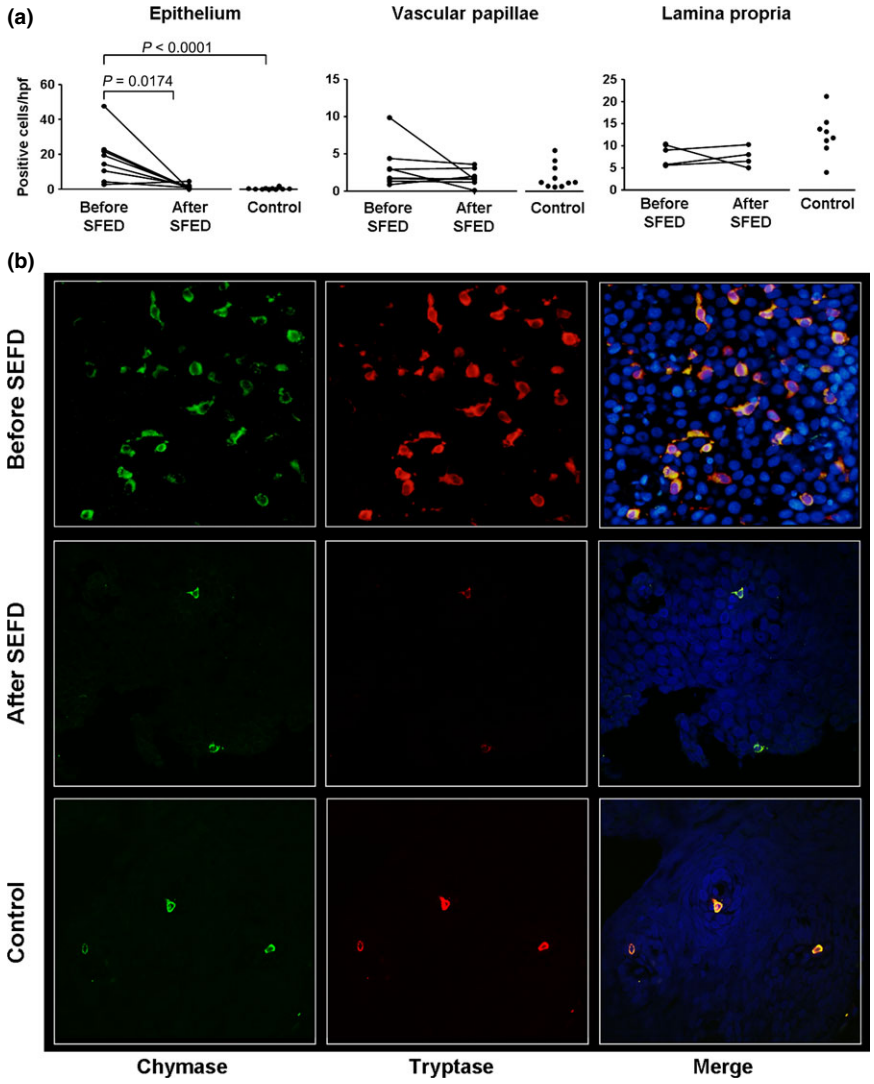

Fig. 4. Histological evaluation of mast cells in the oesophageal mucosa. (a) Individual cell counts per hpf of chymase-positive cells in the epithelium, vascular papillae, and lamina propria of patients with EoE before and after dietary treatment, and in the control group. (b) Representative images of the double immunofluorescence for chymase and tryptase staining in the three experimental groups. Chymase-positive mast cells infiltrate the epithelium in EoE. Dietary treatment reduced cell density, and positive cells were then mainly detected in the vascular papillae. Note: SFED: six-food elimination diet.

noting that three of the 10 patients in our study showed no allergic background, and no differences were noted regarding mast cell counts or activation between atopic and non-atopic patients. This suggests that IgE is not the principal trigger of mast cell activation in EoE. In fact, MC_{TC} are also strong responders to non-IgE-mediated regulatory stimulus including the activation of tolllike receptors [39] or non-immunological mechanisms [48, 49]. The latter include exposure to GER acid [50–52], bile acids [53], or immune mediators, as well as enteric nervous system activation [54]. Among these IgE-independent mechanisms for mast cell activation, one of the most relevant is the ability of certain eosinophil-derived proteins, mainly major basic protein (MBP), to induce mast cell degranulation in an especially attractive, albeit hypothetical, mast cell/eosinophil interaction [55]. In fact, a direct relationship between the density of eosinophils and mast cells has been demonstrated both in our research and in previous reports [21, 25]. Mast cell density (as determined through cell counts in either tryptase, chimase or carboxypeptidase A3-positive cells) directly correlated with oesophageal symptoms in our 10-patient series; we also found a direct association with gene expression levels of the same genes.

Recent advances have provided a plausible explanation for the ability of certain dietary components to initiate and promote EoE, independent of the primary

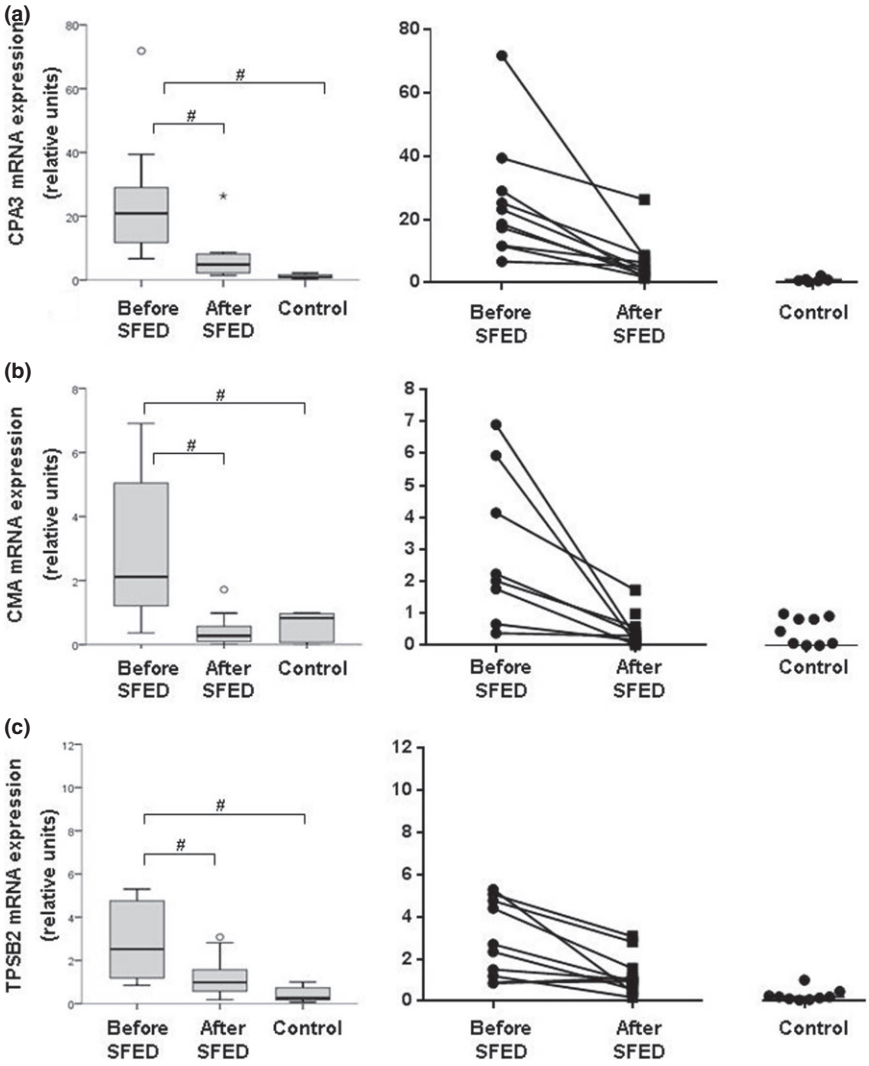

Fig. 5. Gene expression levels of the major mast cell-characteristic proteases in patients with EoE (at baseline and after six-food elimination diet [SFED]-induced remission), and in control subjects. Median and interquartile ranges are represented in the boxes, with whiskers (vertical lines) extending to a limit of ± 1.5 interquartile range. Individual changes in cytokine gene expression are provided. Horizontal bars represent means. (a) carboxypeptidase A-3 (CPA3); (b) chymase (CMA); and (c) tryptase/TPSB2). #Statistically significant differences ($P < 0.05$) before and after treatment in EoE patients compared with controls.

effect of IgE-mediated reactions [56]. Epithelial cells have been shown to have an increasing role as major effectors in initiating EoE, both through recruiting iNKT cells (a major cytokine source) towards the oesophageal epithelium, and through the release of eotaxin-3 and other chemoattractants [57, 58]. Epithelial- and mesenchymal-released TSLP is a key regulator for which a connecting role between the adaptive and innate mucosal-associated immune response has been suggested [47, 59]. In any case, the definitive exclusion of a putative role for IgE-promoting, mast cell-dependent, immediate reactions would require evidence of mast cell activation just after challenging a patient with a known food trigger for EoE, and this has yet to be demonstrated.

Our study is, to the best of our knowledge, the first to find a direct relationship between oesophageal symptoms and gene expression levels of mast cell proteases in adult EoE. Several oesophageal motor disturbances have been identified in patients with EoE by means of manometry, suggesting smooth muscle dysfunction as the origin of symptoms [60]. The ability of mast cells to induce dysmotility and visceral hyperalgesia has been repeatedly documented in several gastrointestinal inflammatory disorders [61–63], including EoE [18]. Indeed, increased mast cell counts are common in the smooth muscle of patients with EoE and have been shown to promote oesophageal smooth muscle contractility *in vitro* [18], although they decrease after topical corticosteroid therapy.

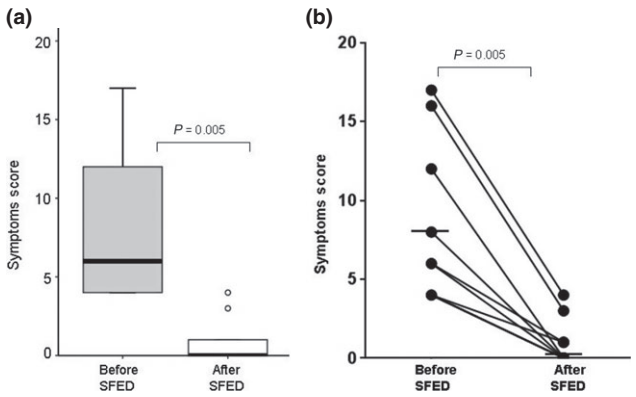

Fig. 6. Score of oesophageal symptoms in patients with EoE: patients at basal conditions and after six-food elimination diet (SFED)-induced histological remission, using the symptom score elaborated by Zaninotto et al. [40] for achalasia. (a) Medians and IQRs are represented in the boxes, with whiskers (vertical lines) extending to a limit of ±1.5 IQRs. (b) Individual changes in symptom score induced by an SFED in patients with EoE. Bars represent means.

Table 3. Relationship between mast cells, eosinophils, gene expression, and clinical symptoms score

	Spearman's rho	*P*
Mast cell peak count – Carboxypeptidase 3	0.61	0.004
Mast cell peak count – Chymase	0.48	0.043
Mast cell peak count – Tryptase	0.47	0.038
Mast cell peak count – Symptom Score	0.78	< 0.001
Carboxypeptidase 3 – Symptom Score	0.67	0.001
Chymase – Symptom Score	0.62	0.006
Tryptase – Symptom Score	0.44	0.049
Mast cell peak count – Eosinophil peak count	0.80	< 0.001
Eosinophil peak count – CCL26	0.80	< 0.001
Eosinophil peak count – Symptom Score	0.89	< 0.001
CCL11 – Symptom Score	0.45	0.045
CCL26 – Symptom Score	0.71	0.001
SCF – Symptom Score	0.39	0.085

The ability of dietary therapy in the form of food restriction to modify the gene expression of mast cells in the oesophageal mucosa of adult patients with EoE had previously only been assessed in a series of six adults [64]. The study found that CPA-3 expression directly correlated with that of eotaxin-3, both of which decreased after food elimination, but increased again during a food reintroduction protocol which led to disease recrudescence. Unfortunately, the researchers did not assess changes in mast cell counts. Our work thus validates previous results and provides additional evidence regarding the regulatory pathways underlying the complex relationship between eosinophils and mast cells.

One strength of our study is that it is the only one to include patients with EoE at the moment of diagnosis; thus, the subjects had no previous exposure to topical steroids or any other anti-inflammatory drugs. As such, the baseline cell densities and gene expression levels obtained can be considered a true reflection of the pathophysiological changes associated with EoE. Additionally, we have determined gene expression for mast cell-related genes by means of real-time PCR in parallel with an examination of protein expression through immunofluorescence staining, finding both to be associated with eosinophil density and symptom score.

Nevertheless, our study has several limitations. The small sample size (only 10 subjects per group) is a result of the difficulty in recruiting patients naïve to EoE therapies who also responded to an SFED. However, the strong associations between cell infiltration, gene expression levels, and oesophageal symptoms score observed in our series make us confident that the results are sufficiently strong and meet our study goals. Another limitation is that while our control group included individuals matched with patients with EoE by gender, the controls were significantly older. This is due to the fact that, according to current guidelines for managing dyspeptic symptoms, endoscopic exams can be avoided in young patients who do not present alarm symptoms. Instead, the standard strategy is to test for *Helicobacter pylori* infection through the urea breath test and then direct treatment [65]. In this sense, the difficulty in recruiting younger individuals undergoing endoscopic exams prevented us from completely matching the age of both groups. One final limitation worth mentioning is that we used a score for evaluating EoE-associated symptoms that had not actually been validated for EoE, but for achalasia. In fact, a number of scales have been used to measure oesophageal symptoms in EoE [36, 66, 67] as a validated tool for clinical assessment is still lacking [68]. In any case, our symptoms scale, which is based on the intensity and frequency of different oesophageal symptoms, has proved reliable and accurate in evaluating variations among individual patients.

In conclusion, our study characterized most oesophageal mast cells as MC_{TC}, which play a relevant role in the pathophysiology of EoE and its associated symptoms. It also documented the efficacy of dietary treatment in reversing the increased density and activity of these cells. Future studies should define the exact mechanisms of mast cell activation and their complex interactions with other inflammatory cells in the pathophysiology of EoE.

Acknowledgements

This work was partially supported by grants from The Castilla-La Mancha Health Research Foundation (*Fundación para la Investigación Sanitaria de Castilla-La Mancha* or FISCAM, grant PI-2010/038 to AJL); the

Health Research Fund of the Carlos III Health Institute (*Fondo de Investigación Sanitaria Instituto de Salud Carlos III*); the General Deputy General Council on Health Research (*Subdirección General de Investigación Sanitaria*), Ministry of Economy and Competitiveness (CP10/00502, PI13/00935 to MV); and the Spanish Society of Digestive Pathologies (*Sociedad Española de Patología Digestiva*), to MV.

Conflicts of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical characteristics of control subjects included in the study.

Table S2. Genes included in the study.

Table S1: Clinical characteristics of control subjects included in the study. Sex: M, male; F, female. Symptoms: Py, pyrosis; Rf, reflux; D, diarrhoea; AcR, acid regurgitation; AP, abdominal pain; Ht, heartburn; WL, weight loss. Endoscopy: N, normal; Atopy: BA, bronchial asthma; AR, allergic rhinitis; DS, drug sensitivity. ND, not determined

Control subject	Age (years)	Sex	Reason for endoscopy	Endoscopy		Family background of atopy	Personal background of atopy
				Calibre	Mucosal appearance		
1	70	F	Py	N	N	ND	ND
2	63	M	Rf	N	N	ND	ND
3	30	M	D	N	N	D	BA, AR
4	22	F	Ht	N	N	No	No
5	41	M	AcR	N	N	ND	ND
6	65	M	AP	N	N	No	No
7	80	M	D	N	N	ND	ND
8	61	M	AP	N	N	ND	AR
9	66	M	D	N	N	No	DS
10	32	M	D	N	N	No	No

Table S2: Genes included in the study.

Gene symbol	Gene name	Accession No.	ABI Gene Expression Assay No.
CPA3	Carboxypeptidase A3, mast cell	NM_001870.2	Hs00922059_m1
CMA	Chymase, mast cell	NM_001836.2	Hs00156558_m1
TPSB2	Tryptase beta 2	NM_024164.5	Hs02576518_gH
CCL11	Chemokine (C-C motif) ligand 11	NM_002986.2	Hs00237013_m1
CCL24	Chemokine (C-C motif) ligand 24	NM_002991.2	Hs00171082_m1
CCL26	Chemokine (C-C motif) ligand 26	NM_006072.4	Hs00171146_m1
CCR3	Chemokine (C-C motif) receptor 3	NM_178329.2	Hs99999027_s1
TGF-β	Transforming growth factor beta	NM_000660.4	Hs99999918_m1
SCF	Stem cell factor	NM_000899.4	Hs00241497_m1
SCFR	Stem cell factor receptor	NM_000222.2	Hs00174029_m1

Artículo 3: Toll-like receptors-mediated pathways activate inflammatory responses in the esophageal mucosa of adult eosinophilic esophagitis.

Clin Transl Gastroenterol. 2018 Apr 25;9(4):147.
doi: 10.1038/s41424-018-0017-4.
***JCR Factor de Impacto: 4,621**;*
*Categoria: Gastroenterology & Hepatology 15/80 (**Q1**)*

Arias et al. *Clinical and Translational Gastroenterology* (2018)9:147
DOI 10.1038/s41424-018-0017-4

Clinical and Translational Gastroenterology

ARTICLE Open Access

Toll-like receptors-mediated pathways activate inflammatory responses in the esophageal mucosa of adult eosinophilic esophagitis

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Abstract

Objectives: Esophageal microbiota and regulation of adaptive immunity are increasingly being investigated in eosinophilic esophagitis (EoE). Toll-like receptors (TLRs) play a central role in the initiation and maintenance of innate immune activity. Our objective was to characterize the esophageal and duodenal innate immune response in EoE and its modulation by dietary therapy.

Methods: Esophageal and duodenal biopsy samples were collected from 10 adults with untreated EoE, before and after effective treatment with a six-food elimination diet (SFED), and 10 controls with normal esophagus. In all cases, bacterial load (by mRNA expression of 16S), TLRs, mucins, transcription factors, interleukins, components of the NKG2D system, and innate immunity effectors were assessed by qPCR. Protein expression of TLRs were also determined by immunofluorescence.

Results: Bacterial load and TLR1, TLR2, TLR4, and TLR9 were overexpressed on biopsies with active EoE compared with controls. Muc1 and Muc5B genes were downregulated while Muc4 was overexpressed. Upregulation of MyD88 and NFκB was found together with IL-1β, IL-6, IL-8, and IL-10 mediators and PER-1, iNOS, and GRZA effectors. NG-K2D components (KLRK1, IL-15, MICB) were also upregulated. In all cases, changes in active EoE were normalized following SFED and mucosal healing. Duodenal samples also showed increased expressions of TLR-1, TLR-2, and TLR-4, but not 16S or any other mediators nor effectors of inflammation.

Conclusions: Esophageal TLR-dependent signaling pathways in EoE support the potential implication of microbiota and the innate immune system in the pathogenesis of this disease.

Introduction

Eosinophilic esophagitis (EoE) is a chronic, food-triggered, immune-mediated disease of the esophagus, clinically characterized by symptoms referred to esophageal dysfunction, and histologically defined by an eosinophil-rich inflammation of the esophageal mucosa[1,2], among other cell types[3]. The incidence and prevalence of EoE have rapidly increased in children and adults in recent

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years[4], making it today a common cause of chronic dysphagia and food impaction in young patients.

The involvement of an adaptive Th2-type immune response to food antigens in EoE was known from the first descriptions of the disease;[5,6] several cytokines, and chemokines derived from T cells present within the inflammatory infiltrate in EoE promote food-specific responses[7,8], in which local production of IgE[9], but also IgG4 derived from plasma cells located in the esophageal lamina propria of EoE patients[10] might play a relevant role. Profibrogenic factors released by inflammatory cells determine fibrous remodeling of the esophageal tissues[11,12]. Avoiding the consumption of specific food triggers, whenever possible, constitutes a first-line therapy for EoE[13,14].

In contrast to the highly specialized adaptive immunity, the innate immune system recognizes and responds to environmental insults and pathogens without the need for an immunoglobulin-driven antigen-specific response. Evidence pointing towards a potential role for the innate immunity in EoE has arisen recently. Esophageal epithelial cells have been revealed as major effectors initiating the inflammatory phenomena in EoE, not just through the release of eotaxin-3 and other chemoattractants for eosinophils[15], but also by promoting the recruitment of invariant natural killer T (iNKT) cells toward the esophageal epithelium[16], which constitutes a major cytokine source. A specific role for mast cells (MCs) has also been recognized in the pathophysiology and symptoms of EoE which reverse after effective dietary treatment[17]. Changes in the esophageal microbiome composition in adult and pediatric EoE patients compared to non-EoE controls have also been recently described[18,19] while modification of the microbiota caused by antibiotic consumption has been recognized as an early life risk factor for developing EoE[20]. Together, these evidences give rise to a potential role that the innate immune system in general, and the microbial pattern recognition receptors (PRRs) in particular, might play in EoE pathogenesis.

Among PRRs, Toll-like receptors (TLRs) are type-I transmembrane receptors expressed both on epithelial and lamina propria cells with the capacity to distinguish between pathogen and commensal microbes[21]. In humans, there are a total of 11 different TLR (named from TLR-1 to TLR-11), each having different specificities which, once stimulated, activate intracellular signal transduction pathways mediated by MAP kinases and NF-κB, ultimately triggering a pro-inflammatory immune response. As a part of the innate immune system[22], TLRs activation is responsible, among other functions, for triggering inflammatory responses by acting as a link between innate and adaptive immunity[23,24]. Indeed, activation and maturation of antigen-presenting cells and regulatory T cells (Tregs) depends on TLR-mediated signaling, highlighting their role on mucosal immune homeostasis.

Numerous studies have evaluated the role of TLRs in inflammatory, autoimmune, and allergic diseases, with the relationship between allergy and TLR activation currently positioned at the frontier of immunology research[22,25,26]. TLR expression in esophageal epithelial samples, however, has only been demonstrated recently[27]. Despite this, no study has yet assessed their potential role in EoE. Therefore, in order to get a deeper insight into this mechanism in the context of EoE, here we have characterized the expression of human TLRs, as well as of several immune-mediators and effectors, on esophageal and duodenal samples from healthy controls and patients with EoE, both before and after dietary-induced disease remission.

Methods

Participants and clinical assessment

Adult EoE patients who were naïve to topical or systemic steroids and dietary therapy for EoE were prospectively recruited. Diagnosis for EoE was defined by consensus guidelines[28] and consisted in (i) infiltration of esophageal epithelium by 15 or more eosinophil leukocytes per high-powered field (hpf) (ii) absence of eosinophilic infiltration in biopsy specimens from gastric and duodenal mucosa; (iii) lack of histologic response after an 8-week trial of PPI therapy; and (iv) exclusion of drug intake, parasites, esophageal caustications, hematologic neoplasm, or other events in the patient's medical history as possible causes of esophageal eosinophilia. Esophageal biopsies were obtained from each patient with EoE at baseline and after 6-weeks of an empiric six-food elimination diet (SFED) that induced histologic and clinical remission of EoE. Patients' support was provided as previously described[31]. The duration and intensity of dysphagia events, along with the frequency and intensity of heartburn and regurgitation, were assessed structurally, by means of a non-validated score developed for achalasia[32] and previously used in adult EoE[12,17], at the beginning of the study and after completing the dietary treatment.

Gender-matched control samples were obtained from individuals who consecutively underwent endoscopy under sedation during the study period, because of dyspepsia or a suspected gastroduodenal ulcer. All selected control subjects exhibited a normal endoscopic appearance of the esophagus, in which hiatus hernia, incompetent cardias, and esophageal peptic lesions were excluded, and the analyses of esophageal mucosal biopsies were also reported as normal. Familial and personal background of atopy was identified in all EoE patients and control participants, based on clinical records.

Endoscopic and biopsy-sampling procedure

All endoscopic exams were performed under propofol sedation by a single board-certified gastroenterologist (AJL) with a flexible 9-mm-caliber Pentax EG-2770K gastroscope (Pentax of America, Inc, Montvale, NJ). A minimum of four biopsies were taken from both upper and lower esophageal thirds with the aid of a standard needle biopsy forceps (Endo Jaw FB-220U, Olympus Medical Systems, Tokyo, Japan). As TLR have been described as overexpressed in the duodenal mucosa of several digestive diseases[29,30], and even in non-inflamed tissues[33], four mucosal biopsies were also taken from the second portion of the duodenum and processed for histopathological analysis. Three additional biopsies from the middle esophageal third and two from the duodenum of each participant were collected during the endoscopic procedure and preserved in an RNA stabilization solution (RNAlater; Ambion, Inc, Austin, Tex) at −80°C until processing for gene expression study.

Histological study

Esophageal samples were fixed in formalin, embedded in paraffin, and routinely processed for hematoxylin and eosin staining. The histological analysis was performed by an experienced pathologist (JMO) blind to the experimental groups. The peak number of eosinophils was counted in the most densely inflamed areas with the aid of Nikon Eclipse 50i (Nikon Corp, Tokyo, Japan) light microscopy in three high-powered field (0.238 mm^2). Peak eosinophil count per hpf was calculated in the epithelial strata by averaging the eosinophil counts.

Immunofluorescence

Formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm. Cuts were deparaffinized and rehydrated following general procedures. Specific antigen retrieval and permeabilization processes were performed depending on the antibody. After treatment with Blocking Solution (Dako Diagnósticos, Barcelona, Spain) for 2 h at room temperature, samples were incubated with the primary antibodies anti-TLR1, TLR2, TLR3, TLR4, TLR6, or TLR9 (Supplementary Table 1) overnight at 4 °C. Incubation with the secondary antibodies Alexa Fluor 594 goat anti-rabbit IgG or Alexa Fluor 488 goat anti-mouse IgG (Life Technologies, Madrid, Spain) was performed for 30 min at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The negative control slides followed the same procedure excluding the addition of the primary antibodies. Fading was controlled using the Prolong anti-fade mounting media (Molecular Probes, Barcelona, Spain). A fluorescence microscope (BX61, Olympus, Barcelona, Spain) was used for visual analysis and images of the epithelium and the lamina propia were taken at high magnification (×400).

Analysis of RNA expression

Total RNA was isolated with the MirVanaTM miRNA Isolation Kit (Ambion), following the manufacturer's instructions. Gene expression for the different determined genes was evaluated in all samples. Each assay and its assay ID number is available at Applied Biosystems (Madrid, Spain) (Supplementary Table 2). Simultaneous quantitative real-time PCRs (qPCR) were performed with TaqMan Low-Density Arrays (Applied Biosystems) preconfigured in a 384-well format and spotted on a microfluidic card. Each TaqMan Gene Expression Assay consists of a forward and reverse primer at a final concentration of 900 nM and a Taq-Man MGB probe (6-FAM dye-labeled; Applied Biosystems), with a final concentration of 250 nM. The assays are gene specific and have been designed to span an exon–exon junction. Thermal cycling conditions were 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min in an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). This procedure was replicated twice for each gene and each sample, with water as a negative control.

Relative changes in mRNA expression of human genes were calculated with the cycle threshold (Ct) method[35] with the aid of Sequence Detection System 2.1 software (Applied Biosystems). The amount of mRNA for each gene was calculated in each sample using the Ct value. Relative gene expression was calculated as follows: $2\Delta\Delta Ct$, where $\Delta\Delta Ct = \Delta Ct_{target\ gene} - \Delta Ct$ control genes. The fold change for the treatment was defined as the relative expression compared with the corresponding control and was calculated as follows: $2\Delta\Delta Ct$, where $\Delta\Delta Ct = \Delta Ct_{patient} - \Delta Ct_{healthy}$, and expressed as arbitrary relative units (rU). Expression levels of all target genes were normalized to 18S, GAPDH, PGK1, GUSB, and b-actin expression.

Bacterial load was determined by using two primers developed against the V4 region of the 16S rRNA, as previously described[34]. Three replicas were amplified per sample and expression levels were normalized to those of the same eukaryotic genes, thus making them independent of the biopsy size.

In order to identify overlap or cluster formation we performed Principal Component Analysis (PCA) plots and Heatmaps by ClustVis web tool[36]. ClustVis is written using the Shiny web application framework (R package version 0.10.2.1) for R statistics software, using several R packages internally[36,37]. Each PCA was calculated using Singular Value Decomposition (SVD) with imputation[38]. Heatmap is plotted using pheatmap R package (version 0.7.7). The package uses popular clustering distances and methods[39] implemented in *dist* and *hclust* functions in R. Heatmaps show a data matrix where coloring gives an overview of the numeric differences, and genes and samples are clustered hierarchically.

Table 1 Clinical characteristics of EoE patients included in the study

Patients	Age (years)	Sex	Time of evolution (months)	Symptoms	Endoscopy		Familiar background of atopy	Personal background of atopy	Identified food triggered
					Caliber	Mucosal appearance			
1	25	M	12	FI, Dy	N	LF, Rg	No	No	F&S & Ri
2	18	M	60	FI, WL	N	LF, C	Sister: D	AR	Le, Nu & Co
3	38	M	4	Dy, AP	R	WP, Rg	No	BA, AR	Mi, Eg, F&S, Le & So
4	36	M	36	FI	N	LF, WP, Rg	Brother: FS	BA, AR	Mi, Nu & So
5	38	F	60	FI, Dy	N	LF, WP	Sister: AR	BA, AR	Wh, Leg & Nu
6	18	M	24	AP, V	N	LF, Rg	No	AR, FS	Mi, Le, Nu & Co
7	51	F	24	FI, Dy	N	LF, WP, Rg	No	No	Mi & Egg
8	34	M	48	FI, Dy, Ht	R	LF, WP, C, Rg	Father: BA; Brother: AR	No	Mi
9	38	M	120	FI, Dy	N	Normal	No	BA, AR, FS	Ri
10	35	M	120	Dy, AP	N	Rg, C	Brother: DS	BA, AR, FS	Mi, F&S & Co

Sex: *M* male, *F* female. Symptoms: *FI* food impaction, *Dy* dysphagia, *AP* abdominal pain, *V* vomiting, *Ht* heartburn, *WL* weight loss. Endoscopy: *N* normal, *R* reduced, *Rg* rings, *LF* longitudinal furrows, *C* crêpe-paper appearance, *WP* white plaques. Atopy: *BA* bronchial asthma, *AR* allergic rhinitis, *FS* food sensitivity, *D* dermatitis, *DS* drug sensitivity. Food triggers: *Mi* milk, *Ri* rice, *F&S* fish & seafood, *Le* legumes, *Nu* nuts, *Wh* wheat, *Co* corn, *Eg* eggs, *So* soy

Statistical analysis

Optimal sample size was calculated based on our previous results[17] aimed for a power of 90%. Means and standard deviations were reported for continuous variables and are expressed as mean (standard deviation) throughout the text. Proportions were reported for categorical data. Results are expressed as a median with an interquartile rank (IQR) for scoring clinical symptoms. Comparisons between groups (control subjects and EoE patients) were performed with nonparametric tests: the Mann–Whitney *U*-test for quantitative variables and the Fisher exact test for nominal variables. For comparison before and after SFED treatment, the nonparametric-paired Wilcoxon signed-rank test was used. To control for multiple testing, post hoc comparisons were performed using Holm-Bonferroni-corrected p values. A nonparametric correlation test (Spearman's rho) was used for analyzing the association between eosinophils, gene expression, and clinical symptoms. A 0.05 level of significance was used throughout. Statistical analyses were performed with the aid of PASW 18.0 statistical analysis software (SPSS Inc, Chicago, Ill).

Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board of La Mancha Centro General Hospital. Informed consent was obtained from all patients prior to all endoscopic exams.

Results

Study population

Of the 14 patients with EoE screened, 10 (8 men and 2 women) achieved histological and clinical remission and were included in this study. Additionally, 10 gender-matched control subjects were also included. The groups had a mean (standard deviation) age of 33.1 (10.1) and 53 (19.9) years, respectively. Individual clinical characteristics of the experimental subjects are given in Table 1 and Supplementary Table 3. Mean duration of symptoms in EoE patients exceeded 4 years (50.8 ± 40.9 months). No difference in clinical manifestations was observed between atopic and non-atopic subjects (Table 2).

Intraepithelial eosinophils

In EoE patients, absolute peak intraepithelial eosinophil density was 56.8 (29.9) cells/hpf, which decreased to 3 (4.2) cells/hpf after SFED-based treatment ($p < 0.001$). No eosinophils were detected in any of the esophageal samples from controls. No differences in eosinophil counts were detected between atopic and non-atopic EoE patients, being 55 (30.4) vs. 61 (34.8) cells/hpf, respectively. No eosinophilic infiltration was found in duodenal samples.

Table 2 Clinical characteristics and gene expression levels of atopic and non-atopic EoE patients

	Atopic vs. non-atopic	*p*
Time of evolution (months)	60.6 (45.1) vs. 28 (18.3)	0.250[a]
Symptom score	9 (5.9) vs. 6 (2)	0.723[a]
Symptoms		
Dysphagia	57.1% vs. 100%	0.475[b]
Food impaction	57.1% vs. 100%	0.475[b]
Abdominal pain	42.9% vs. 0%	0.475[b]
Heartburn	0% vs. 33.3%	0.300[b]
Vomiting	14.3% vs. 0%	>0.999[b]
Weight loss	14.3% vs. 0%	>0.999[b]
Endoscopy findings		
Reduced caliber	14.3% vs. 33.3%	>0.999[b]
Normal mucosa	14.3% vs. 0%	>0.999[b]
Longitudinal furrows	57.1% vs. 100%	0.475[b]
Rings	71.4% vs. 66.7%	>0.999[b]
Crêpe-paper appearance	28.6% vs. 33.3%	>0.999[b]
White plaques	42.9% vs. 66.7%	>0.999[b]
Peak eosinophil count	55 (30.4) vs. 61 (34.8)	0.908[a]
TLR1 gene expression	2.2 (2.3) vs. 1.5 (2.4)	0.569[a]
TLR2 gene expression	41.3 (62.9) vs. 44.2 (34.5)	0.909[a]
TLR3 gene expression	10.5 (9.5) vs. 3.2 (4.4)	0.087[a]
TLR4 gene expression	3.6 (9.3) vs. 3.1 (2.7)	0.569[a]
TLR6 gene expression	2.1 (4.6) vs. 2.9 (3.1)	0.909[a]
TLR9 gene expression	1.9 (6.7) vs. 3.8 (2.7)	0.425[a]
16S gene expression	0.75 (0.5) vs. 0.54 (0.8)	0.732[a]
MUC1 gene expression	1.1 (1.7) vs. 0.3 (0.9)	0.360[a]
MUC2 gene expression	NA	—
MUC4 gene expression	14.4 (22.1) vs. 20.3 (10.6)	0.732[a]
MUC5B gene expression	0.32 (0.51) vs. 0.09 (0.03)	0.138[a]
MyD88 gene expression	2 (0.4) vs. 2.3 (0.5)	0.425[a]
NF-κB gene expression	2.9 (0.8) vs. 4 (0.8)	0.305[a]
IL-1α gene expression	0.13 (0.17) vs. 0.13 (0.06)	0.909[a]
IL-1β gene expression	3.3 (5.6) vs. 8.8 (4.3)	0.125[a]
IL-6 gene expression	0.21 (0.14) vs. 1.3 (1.2)	0.138[a]
IL-8 gene expression	103 .7 (196.6) vs. 179.39 (138.1)	0.425[a]
IL-10 gene expression	6.7 (5.7) vs. 29.9 (14.8)	0.087[a]
TNF-α gene expression	1.8 (2.2) vs. 1 (2.2)	0.909[a]
PRF1 gene expression	10.4 (8.7) vs. 7.9 (5.7)	0.909[a]
iNOS gene expression	0.041 (0.03) vs. 0.03 (0.02)	0.909[a]
GZMA gene expression	2.3 (1.7) vs. 3 (4.3)	0.909[a]
GZMB gene expression	17.3 (18.7) vs. 26.3 (13)	0.425[a]
IL-15 gene expression	13.1 (11.8) vs. 15.7 (6.3)	0.305[a]
MICA gene expression	1.2 (1.3) vs. 0.8 (1.6)	0.909[a]
MICB gene expression	2.8 (2.4) vs. 2.5 (0.6)	0.305[a]
KLRK1 gene expression	1.7 (1.2) vs. 4.6 (4.4)	0.210[a]

[a] Mann–Whitney *U*-test
[b] Chi-square test

TLR1, TLR2, TLR4, and TLR9 are overexpressed on the inflamed EoE esophagus

Given that it has been recently demonstrated that TLRs are expressed on esophageal epithelial cells[40], here we decided to assess their levels on the inflamed mucosa from EoE patients, as well as on the paired non-inflamed mucosa from the same patients after dietary treatment-induced disease remission compared with healthy controls. Our results, showed that mRNA expression of 4 out of the 6 TLR studied was higher in patients with active EoE, compared to healthy controls: TLR1 (2.7-fold increase), TLR2 (3.7-fold increase), TLR4 (4.6-fold increase), and TLR9 (3.4-fold increase) ($p < 0.05$ for all comparisons). TLR expression in EoE patients returned to normal following dietary therapy-induced remission (Fig. 1a–f) ($p < 0.05$ regarding baseline conditions), findings confirmed at the protein level by immunofluorescence (Fig. 1g–j). No significant changes were found for TLR3 and TLR6 mRNA or protein expression. No association was observed between age of patients/controls and TLR expression levels (data not shown).

TLR receptors allow the innate immune system to recognize conserved pathogen associated molecular patterns, so we next determined the total mucosa-associated microbiota load in those samples. The average bacterial load detected in esophageal samples of subjects with active EoE was higher (2.85-fold) compared to control non-EoE samples ($p < 0.002$), thus confirming previous observations on a pediatric cohort[18]. Microbiota levels were subsequently normalized (1.16-fold increase) following SFED-induced disease remission ($p < 0.005$) (Fig. 2a), in parallel with the observed TLRs expression.

Given that the microbiota is not usually in direct contact with the epithelium but, instead, embedded on the mucus-layer, we also studied the expression levels of the mucins that have been described to be expressed by the human esophagus[41,42]. Our results revealed that, while Muc1 and Muc5B were downregulated by 2-fold ($p = 0.023$) and 21.5-fold decrease ($p = 0.003$), respectively, Muc4 was expanded on the inflamed mucosa from EoE patients (7.2-fold increase; $p = 0.001$) with all mucin levels

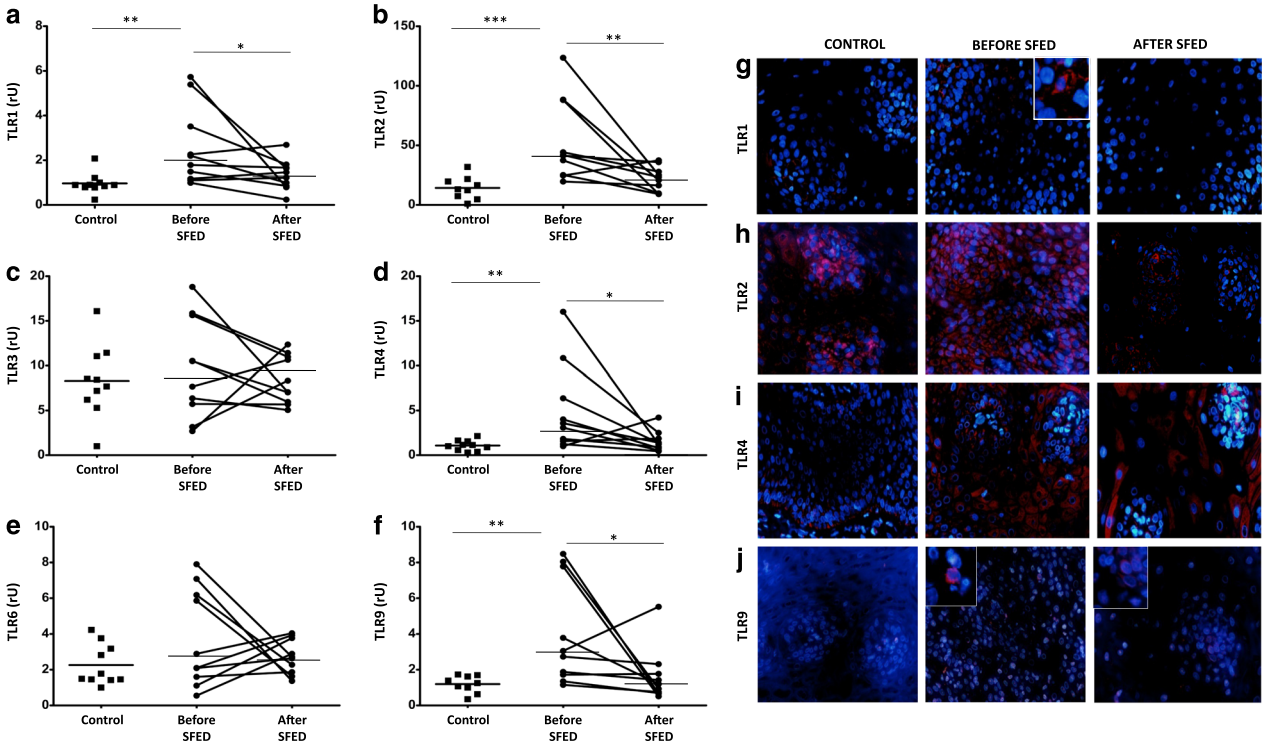

Fig. 1 TLR overexpression in the esophagus of EoE patients. a–f mRNA expression (in relative units) of TLR1, TLR2, TLR3, TLR4, TLR6, and TLR9 in esophageal biopsies from EoE patients before and after six-food elimination diet (SFED) treatment, and healthy controls. **g–j** Immunofluorescence expression of TLR1, TLR2, TLR4, and TLR9 was also determined on the same type of samples. Paired *t*-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired *t*-test. Horizontal bars indicate mean values (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

restored to normal following SFED-induced mucosal healing (Fig. 2b–d). As expected, and in agreement with the literature[42], Muc2 expression was not found in our samples.

The innate immune system is activated in the inflamed mucosa from active EoE patients

Having identified that the microbiota, TLR receptors, and mucins expression were altered in adult patients with active EoE (Figs. 1 and 2), we next studied whether that could translate to an activated innate immune system in those patients. Every TLR—except TLR3 that was not upregulated in our samples (Fig. 1)—utilizes the adapter protein MyD88 to activate the transcription factor NF-κB[43]. Therefore, we first assessed the mucosal expression of both transcription factors (Fig. 3a, b), which were upregulated in samples of EoE patients with active disease (1.8- and 2.2-fold increase, respectively; $p < 0.001$) suggesting that TLR signaling is functional in those patients. In order to further confirm this signaling pathway, we assessed the expression of several NF-κB-induced cytokines. IL-1β (3.5-fold increase; $p < 0.01$), IL-6 (4-fold increase; $p < 0.05$), IL-8 (12.2-fold increase; $p < 0.001$), and IL-10 (6.8-fold increase; $p < 0.001$) were also upregulated on the inflamed mucosa of EoE patients compared to controls, values that returned to normal following SFED-induced mucosal healing, in parallel to MyD88 and NF-κB ($p < 0.001$ in both cases). No changes were noted for IL-1α and TNFα, (Fig. 3c–h).

As a consequence of the immune activation displayed by the esophageal mucosa on EoE patients, we further studied whether these changes also correlated with the expression of several innate immune effectors including PRF-1, iNOS, GZMA, and GZMB (Fig. 4a–d) all of them, with the exception of GZMB, were upregulated in the inflamed mucosa from those patients (1.6-, 7.1-, and 2.7-fold increase, respectively; $p < 0.05$; $p < 0.001$, and $p < 0.05$, respectively) compared to controls, with levels being restored to control values following dietary intervention ($p < 0.05$ compared to baseline).

Finally, we also assessed expression of the NK-G2D system (IL-15, MICA, MICB, and KLRL1)[44], which was also upregulated in the inflamed mucosa from EoE patients (2.8-fold increase for IL-15, 2.6-fold increase for MICB, 2.4-fold increase for KLRK1; $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively) (Fig. 4e–h). The levels of IL-15 and KLRK1 came back to normal following dietary intervention ($p < 0.001$ and $p < 0.05$, respectively). No

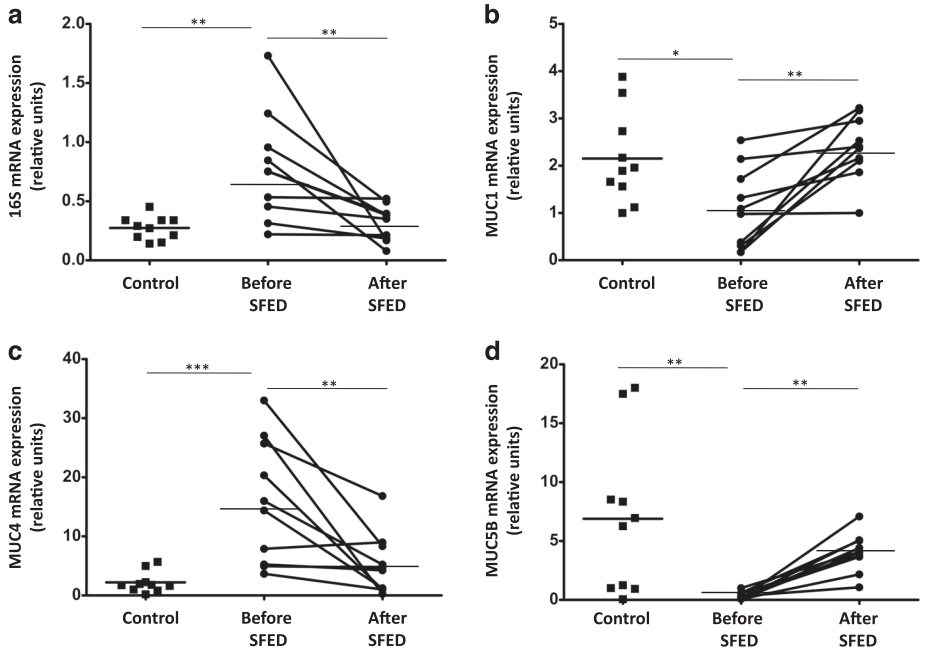

Fig. 2 Bacterial load and mucin expression in the esophagus of EoE patients. a Total microbiota load (determined as 16s gene expression) and **b–d** mRNA expression (in relative units) of Muc1, Muc4, and Muc5B mucins were determined in esophagus biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired *t*-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired t-test. Horizontal bars indicate mean values (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

changes in mRNA expression of MICA were noted. Together, our results confirm that the innate immune system is activated in active EoE patients, hence suggesting that it may participate in its pathogenesis.

Increased duodenal expression of TLR receptors, but not other immune components, in EoE

The esophagus of EoE patients carries a higher bacterial load, which coupled with altered mucus layers and increased levels of TLR receptors (Figs. 1 and 2) results in an activated innate immune system in those patients (Figs. 3 and 4). Therefore, we next studied whether some of those characteristics could also be displayed in other gastrointestinal tissues where EoE patients do not display inflammation, as is the case with the duodenum.

To our surprise, TLR1 (2.04-fold increase, $p = 0.001$), TLR2 (1.4-fold increase; $p = 0.007$), and TLR4 (1.4-fold increase; $p = 0.013$), but not TLR9, were also upregulated in the non-inflamed duodenum from the same patients, levels that were restored to control values in SFED-induced disease remission (Fig. 5a–f). Nevertheless, total bacteria load (Fig. 5g) as well as mucin levels (Supplementary Figure 1) were normal in the same patients. Indeed, the increased expression of duodenal TLRs does not appear to be functional as it did not result in increased levels of transcription factors triggered by TLRs (Supplementary Figure 2A,B), higher cytokine profile (Supplementary Figure 2C-H), levels of innate immunity effectors (Supplementary Figure 3A-D) or the activation of the NK-G2D system (Supplementary Figure 3E-H) on the duodenum.

Differential genetic signature in the esophagus and the duodenum

Having detected an altered gene expression profile in samples from patients with active EoE regarding controls with a healthy esophagus, which decreased following SFED-induced remission, we next studied whether that was reflected in a differential gene expression fingerprint for those patients. Given that TLR expression was also higher in the non-inflamed duodenum of EoE patients, we first analyzed all the data revealing that the samples sort together based on the tissue (Fig. 6) irrespective of the source of the patients, as should be expected, given that the esophagus and the duodenum are two different sections of the gastrointestinal tract with different functions and structures.

We also performed a multivariate analysis separating the samples based on the tissue source. Our results revealed how esophageal samples from patients with active EoE display a differential gene expression profile, compared with the EoE samples under remission and from controls with a healthy esophagus, when studied both as a PCA (Supplementary Figure 4A) or as a

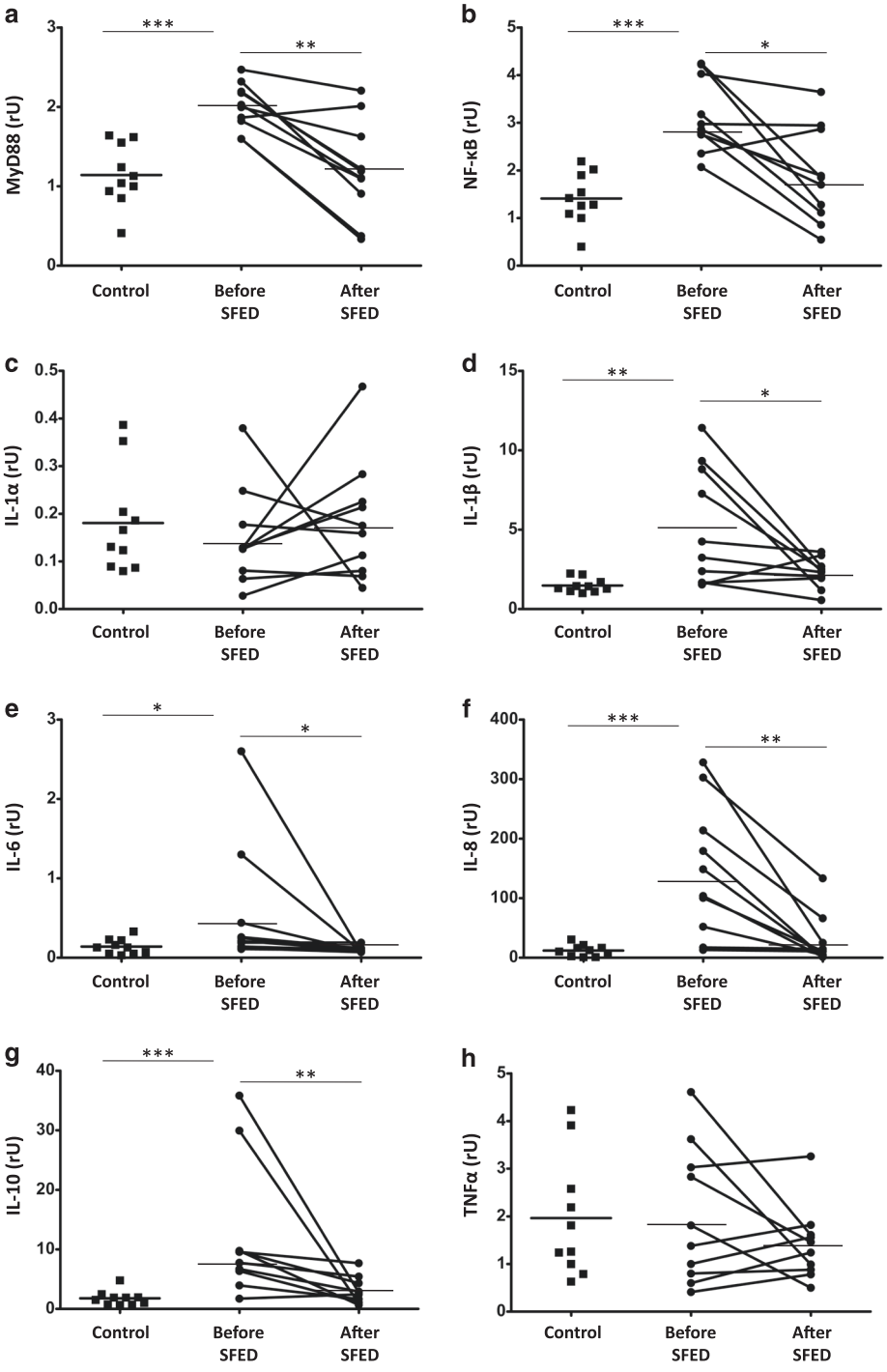

Fig. 3 Innate immune system activation in the esophageal mucosa of EoE patients. a, **b** mRNA of transcription factors (MyD88 and NF-κB) and **c**–**h** cytokines (IL-1α, IL-1β, IL-6, IL-8, IL-10, and TNFα) expression (in relative units) were determined in esophageal biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired *t*-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired *t*-test. Horizontal bars indicate mean values (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

heatmap analysis (Supplementary Figure 4B), although, the same was not true for the duodenum (Supplementary Figure 5). Therefore, although TLR receptors seem to be constitutively overexpressed throughout the upper gastrointestinal tract during active EoE, their signaling is only functional in the esophagus of these patients, hence keeping the immune response restricted to this segment.

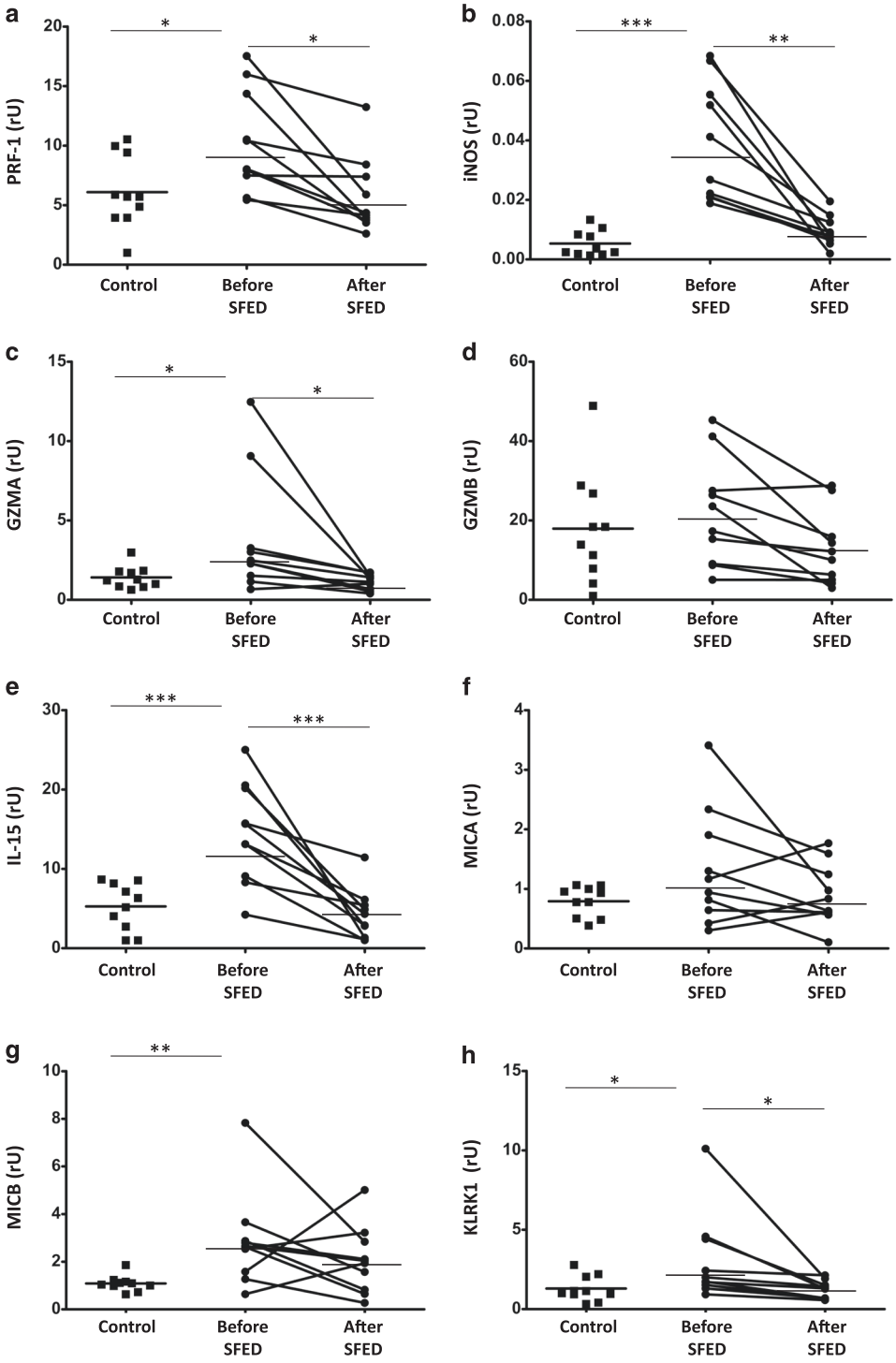

Fig. 4 Activation of the NKG2D system and expression of effectors of inflammation in the esophageal mucosa of EoE patients. a–d mRNA of innate immunity effectors (PRF-1, iNOS, GZMA, and GZMB); and **e–h** the NK-G2D system (IL-15, MIC-A, MIC-B, and KLRK1) expression (in relative units) were determined in esophagus biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired *t*-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired *t*-test. Horizontal bars indicate mean values (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

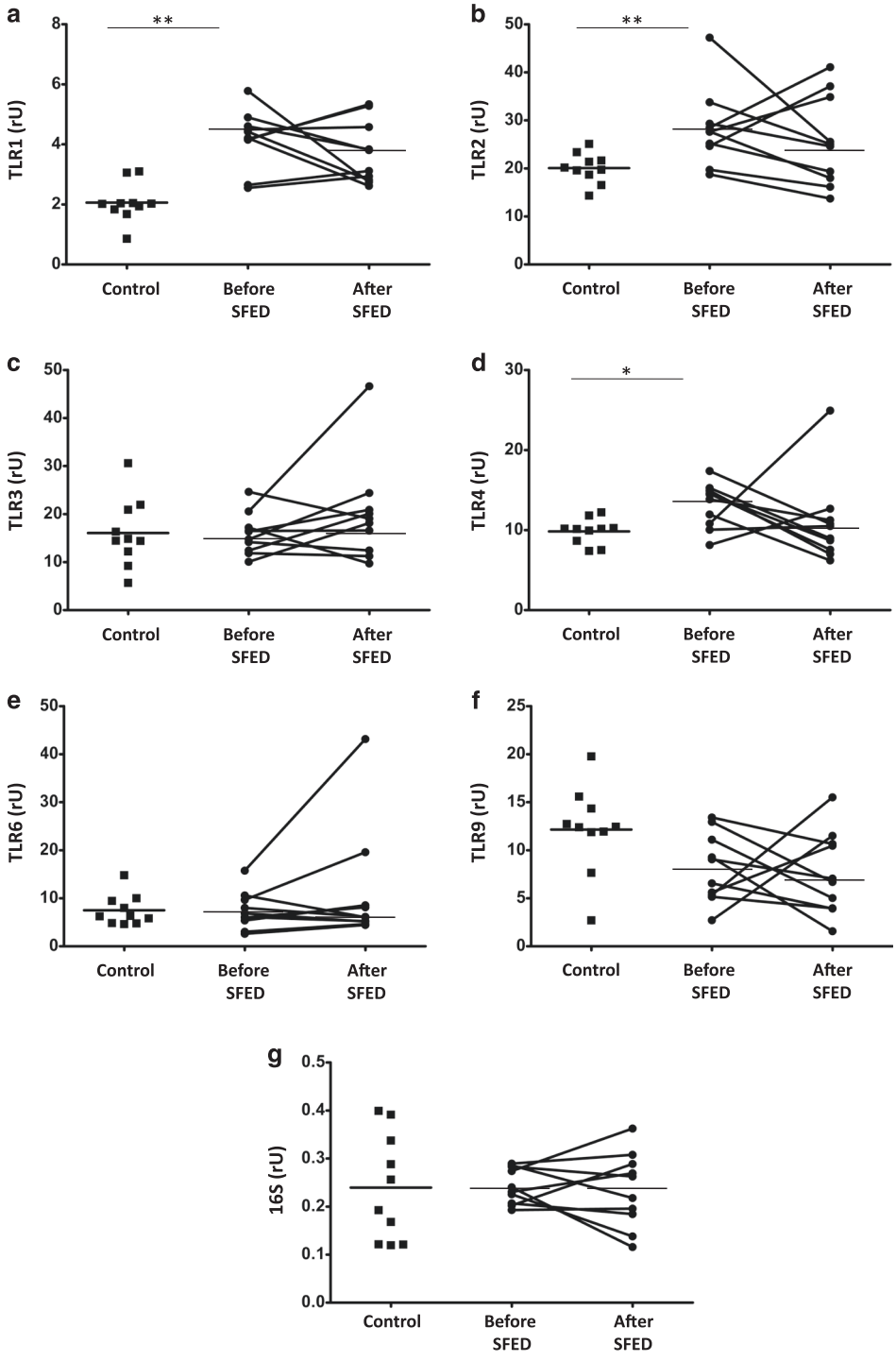

Fig. 5 The TLR overexpression in the duodenum from EoE patients it not coupled with increased bacterial load. a–**f** mRNA expression (in relative units) of TLRs (TLR1, TLR2, TLR3, TLR4, TLR6, and TLR9); and **g** microbial 16S were determined in duodenal biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired *t*-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired *t*-test. Horizontal bars indicate mean values (*$p < 0.05$; **$p < 0.01$)

Discussion

This is the first study examining the potential role of TLRs in the pathophysiology of EoE. Our results demonstrate that active EoE is characterized by upregulated esophageal expression of TLRs compared to healthy controls, despite the wide interindividual variability

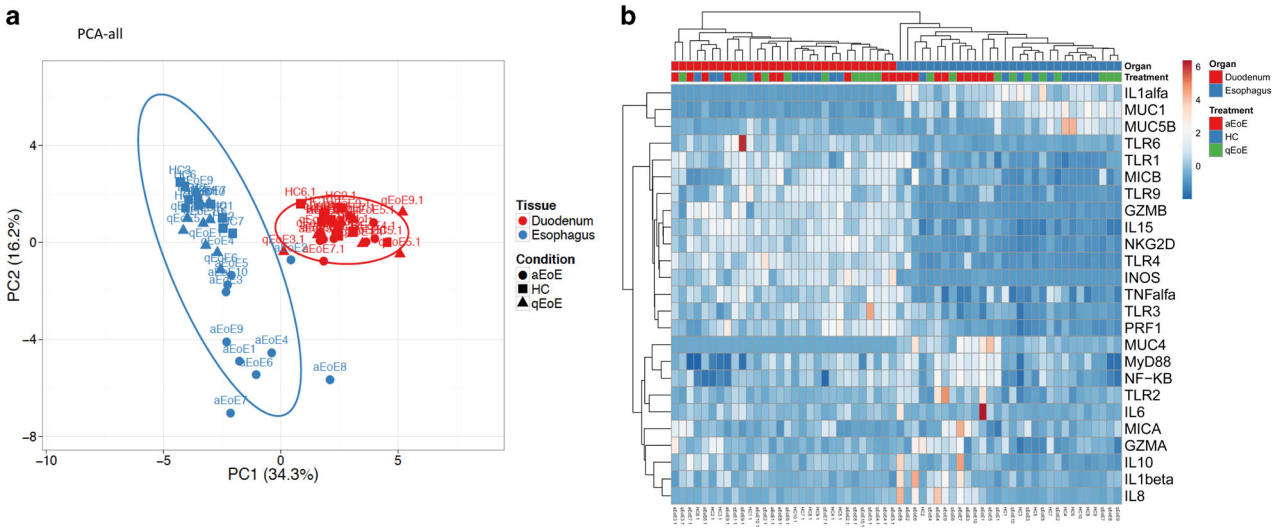

Fig. 6 The esophagus and the duodenum display a differential gene expression profile. a Principal component analysis (PCA) and **b** Heatmaps were determined using all genes detailed in Table 2 from both the esophagus and the duodenum from active (aEoE) and quiescent (qEoE) patients and healthy controls

documented in our series of patients. Moreover, transcription factors and subsequent effectors of the TLRs signaling pathway are also upregulated in EoE, and restored to control after effective dietary treatment. In contrast, the duodenal mucosa shows no inflammatory activity despite comparable profile expression of the same TLR genes. This study adds to the cumulative literature investigating the role of TLRs in different gastrointestinal inflammatory conditions, including inflammatory bowel disease[29,45,46], celiac disease[30,47], food allergy[48], and several atopic disorders[49,50].

In recent years, multiple studies have investigated the signaling pathways mediated by TLRs in the allergic airway disease[51,52], where they regulate immune responses and are connected to the activity of high affinity IgE receptor (FcεRI) expressed on mast cells, acting as a connector between the innate and adaptive immune systems. A predominant role for TLR-2, 4 and 9 has been recognized in bronchial asthma[53,54]. In contrast, the functioning of TLRs in the gastrointestinal tract has just started to be defined and its role is being increasingly recognized in digestive disorders. However, a map regarding TLR expression by different cell types in different human intestinal segments is still missing, something which is particularly important, as the properties of the immune system change systematically throughout the length of the gastrointestinal tract[55]. Indeed, focusing on the esophagus, which is particularly exposed to multiple antigens from microbial, alimentary and airborne origin, this organ requires specific mechanisms to protect its mucosa from chronic damage, including an effective peristaltic activity, epithelial tight junctions, and stratified squamous epithelium. Also, the role of esophageal epithelial cells in immune defense and maintenance of tolerance has not yet been fully investigated.

Bronchial asthma and EoE share multiple resemblances, including an altered Th2-type immune response triggered by potentially innocuous antigens, the involvement of eosinophils and mast cells in the pathophysiology[17], the transmural inflammation that promotes smooth muscle dysfunction and fibrous remodeling[8,56], and clinico-pathological response to topic steroids and avoidance of antigen triggers exposure[13,57,58]. However, and despite all these similarities, as well as the fact that the prevalence of bronchial asthma among EoE patients is three times higher than in the non-EoE population[59], no study has assessed yet the role of TLRs on EoE, as in the case of bronchial asthma[52,53]. Hence, and given that it has been recently reported that TLR receptors are expressed in the healthy esophagus[40,60], we decided to characterize their expression in the context of EoE by describing how TLR-1, TLR-2, TLR-4, and TLR-9 are expanded in the inflamed mucosa from active EoE patients, and its modulation by SFED.

TLR-1 responds to triacyl lipopeptides and TLR-2 to lipotecoic acid and peptidoglycan, both being components of the bacterial wall[61]. Both are involved in reducing the activation of FcεRI[54,62], which results in a reduced IgE-mediated mast cell degranulation. TLR-4, on the contrary, is stimulated upon exposure to lipopolysaccharide present in Gram-negative bacteria. Some allergens (such as the major house dust mite allergen or Derp2) show a structural homology with MD2 protein, which is a co-mediator of TLR-4 activation[54,63], and

could activate TLR-4-mediated response by a molecular mimicry mechanism. In contrast to TLR-1 and 2, stimulation of TLR-4 increases the activation of FcεRI and promotes Th2-type cytokines involved in eosinophilic responses, as documented in respiratory tract allergy[64]. In resting conditions, TLR-4 expression is reduced in bronchial mucosal referred to TLR-2, with the TLR-2/4 ratio determining the final sense of the FcεRI activation[65,66]. In the particular case of our EoE samples, expression of TLR-2 was 10-fold higher that TLR-1, in agreement with the lack of a significant role for IgE in EoE patients. Indeed, IgE plays a limited role in the pathophysiology of EoE, and although it binds to mast cells in the epithelium of atopic patients with this disease[67], it does not constitute its main route of activation[17]. Hence, EoE patients do not develop rapid inflammatory responses following exposure to triggering foods[68], and treatment with anti-IgE monoclonal antibodies has been shown to be completely ineffective improving esophageal symptoms and inflammation in patients with EoE[10]. Finally, TLR-9 is an intracellular receptor activated by bacterial CpG-DNA binding, promoting Th1-type immune responses with increased production of IFNα-b. The stimulation of FcεRI by allergens suppresses the activation of TLR-9, with the consequent reduction of Th1 responses and the promotion of Th2 ones leading to the appearance of allergic reactions.

The increased expression of TLR in active EoE was accompanied by higher bacterial load detected in the same samples and by a downregulation in Muc1 and Muc5B genes, probably determined by epithelial cell damage and dysfunction, impaired mucosal integrity, and increased permeability[69] with exposition to bacterial components, and enhanced activation of the mucosal innate immunity mediated by TLRs, which is restored after avoiding exposure to food antigens. Despite constituting a plausible explanation for our results, some other findings point towards the hypothesis that TLRs may play a primary role in EoE. To begin with, an increased expression of Muc4 gene was found, potentially to compensate for the decrease in Muc1 and Muc5B, which suggests that the mucous integrity in active EoE is preserved enough to limit a direct contact of the esophageal microbiota with the mucosal surface. In addition, signaling pathways specific for TLR activation (IL-8, MyD88), together with increased production of several effectors of direct cytotoxicity (PRF-1, GZMA, iNOS) make it hard to consider TLR activation as an epiphenomenon. Notably, increased TLR expression was also found in the duodenum from the same patients despite having a non-inflamed mucosa (as confirmed both during endoscopy and histological assessment) while displaying no changes in bacterial load or upregulation of mediators of inflammation. All together, our results suggest that TLRs are primarily involved in EoE pathogenesis. It can be speculated therefore that an overexpression of TLRs in non-inflamed segments of the gastrointestinal tract of EoE patients could parallel the increase of proinflammatory cytokines also in non-inflamed tissue from patients with IBD[33]. The question remains, however, why if TLRs are also overexpressed on the non-inflamed mucosa from these patients, disease is nevertheless restricted to the esophagus. One possibility is that increased mucosa-associated microbiota load (or its metabolic activity) in the esophagus (but not in the duodenum) may be the trigger for inflammation (either as a direct effect or by mimicking dietary components), an issue which we are currently studying. Indeed, and given the study approach we used (qPCR), cell types responsible for mucosal TLRs expression were not defined; ongoing work is trying to address this point, by defining the exact immune or epithelial cells that overexpress TLRs, in agreement with recent observations[27,40]. Last, but not least, current work is also characterizing the mucosa-associated microbiota from those patients, with the overall aim of unraveling the specific microbiota contribution to EoE pathogenesis.

We are aware, however, of the limitations from our study, the main one being the limited sample size (only 10 subjects per group) This was as a consequence of the difficulty in recruiting patients who are naïve to therapy and who also responded to a SFED. Despite the significant differences in gene expression levels between EoE and control samples, a wide variability in expression levels from patient to patient was documented, which prohibits a simplified interpretation of the data. The small number of patients included in this study therefore prevented deeper analysis of the source of such variability. We are also aware that our control group included gender but not aged-matched healthy individuals. This is due to the fact that, according to current guidelines for managing dyspepsia, endoscopic exams can be avoided in young patients who do not present alarming symptoms[70]. Nevertheless, we feel that these limitations are lessened by the fact that we have only included patients with EoE at the moment of diagnosis, hence, eliminating the effect of previous exposure to topical steroids or any other anti-inflammatory drugs. As such, baseline eosinophil densities and gene expression levels are a true reflection of the pathophysiological changes associated with EoE.

In summary, we here provide evidence, for the first time to our knowledge, that TLR-dependent signaling pathways are activated in the esophageal mucosa of adult patients with EoE, strongly suggesting a role in the pathophysiology of the disease. The exact mechanisms however that mediate the complex interactions between esophageal microbiota, the innate immune system and food-specific inflammatory responses in the pathophysiology of EoE warrants further research.

Study Highlights

What is current knowledge

- EoE is a predominantly Th2-type inflammatory esophageal condition, in which preliminary evidence points to a potential role for innate immunity.
- The role of TLRs has been evaluated in several inflammatory, autoimmune, and allergic diseases. However, a possible involvement of TLR-mediated signaling in the pathophysiology of EoE has not yet been documented.

What is new here

- Active EoE is characterized by an upregulated expression of TLRs in the esophageal mucosa compared to healthy controls, which returns to normal after dietary therapy-induced remission.
- Activation of TLRs in the esophageal mucosa of patients with EoE supports a relevant role for microbiota in the pathophysiology of the disease.
- TLR-mediated signaling pathways are functional in the esophageal mucosa of patients with active EoE, promoting an activation of the innate immune system that is restricted to the esophagus and contributes to cell damage.

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Competing interests

Guarantor of the article: AJ Lucendo

Specific author contributions: Á.A.: Study conception and design, collection, analysis, and interpretation of data, drafting and revision of the manuscript and approval of the final version of the manuscript. M.V.: Study conception and design, immunofluorescence analysis of esophageal samples, interpretation of data, drafting of the manuscript, and approval of the final version of the manuscript. D.B.: Study conception and design, analysis and interpretation of data, drafting and revision of the manuscript, and approval of the final version of the manuscript. J.M.O.: Histological analysis of esophageal samples, interpretation of data, and approval of the final version of the manuscript. M.F.: Immunofluorescence analysis of esophageal samples, interpretation of data, and approval of the final version of the manuscript. A.M.-A.: Analysis and interpretation of data, and approval of the final version of the manuscript. P.M.-F.: Study conception and design, collection, analysis, and interpretation of data, and approval of the final version of the manuscript. A.M.G.-C.: Immunofluorescence analysis of esophageal samples, interpretation of data, and approval of the final version of the manuscript. T.M.-H.: Histological processing of esophageal samples, interpretation of data, and approval of the final version of the manuscript. L.A.-G.: Interpretation of data and approval of the final version of the manuscript. A.J.L.: Study conception and design, patient diagnosis (performance of endoscopic examinations and esophageal biopsies) and follow-up, interpretation of data, drafting of the manuscript, and approval of the final version of the manuscript.

Financial support: This work was supported by grants from The Castilla-La Mancha Health Research Foundation (Fundación para la Investigación Sanitaria de Castilla-La Mancha or FISCAM) grant PI-2010/038 (A.J.L.); Vall d'Hebron Institut de Recerca, Programa de becas predoctorales "Amics de Vall d'Hebron": PRED-VHIR-2014-018 (M.F.), and Fondo Europeo de Desarrollo Regional (FEDER), Fondo de Investigación en Salud and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas-CIBEREHD, Instituto de Salud Carlos III, Subdirección General de Investigación Sanitaria, Ministerio de Economía y Competitividad: CPII16/00031 and PI16/00583 (M.V.) and CIBEREHD EDH16PI02 (M.V. and D.B.).

Potential competing interests: None to report.

The online version of this article (https://doi.org/10.1038/s41424-018-0017-4) contains supplementary material, which is available to authorized users.

Received: 28 November 2017 Revised: 16 February 2018 Accepted: 22 February 2018
Published online: 25 April 2018

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Supplementary Table E1. Primary Antibodies Used in the Immunohistochemical Study.

Molecule	Antibody		Reference	Dilution	Antigen retrieval	Permeabilization
TLR1	Rabbit polyclonal anti-TLR1	Abcam	37068	1/100	Pepsine 37ºC 15 minutes	NO
TLR2	Rabbit polyclonal anti-TLR2	Abcam	24192	1/100	Tris-EDTA Buffer pH 9, 120ºC 15 min	NO
TLR3	Mouse monoclonal anti-TLR3	Abcam	12085	1/50	Sodium Citrate Buffer pH 6, 120ºC 15 min	0.1% Triton X-100 in PBS
TLR4	Rabbit polyclonal anti-TLR4	Abcam	13556	1/100	Tris-EDTA Buffer pH 9, 120ºC 15 min	NO
TLR6	Rabbit polyclonal anti-TLR6	Thermo	PA5-29697	1/50	Sodium Citrate Buffer pH6, 120ºC 15 min	0.1% Triton X-100 in PBS
TLR9	Rabbit polyclonal anti-TLR9	Abcam	62577	1/100	Tris-EDTA Buffer pH 9, 120ºC 15 min	NO

Supplementary Table E2: Relation of genes analyzed in the research

Gene symbol	Gene name	Accession No.	ABI Gene Expression Assay No.
TLR1	Toll Like Receptor 1	NM_003263.3	Hs00413978_m1
TLR2	Toll Like Receptor 2	NM_003264.3	Hs01872448_s1
TLR3	Toll Like Receptor 3	NM_003265.2	Hs01551077_m1
TLR4	Toll Like Receptor 4	NM_138554.3	Hs01060206_m1
TLR6	Toll Like Receptor 6	NM_006068.4	Hs00271977_s1
TLR9	Toll Like Receptor 9	NM_017442.3	Hs00152973_m1
16S	16S rRNA	Methods as in described in reference #31	
MUC1	Mucin 1	NM_001018016.2	Hs00159357_m1
MUC2	Mucin 2	NM_002457.3	Hs03005103_g1
MUC4	Mucin 4	NM_001322468.1	Hs00366414_m1
MUC5B	Mucin 5B	NM_002458.2	Hs00861595_m1
MyD88	Myeloid differentiation primary response gene 88	NM_001172566.1	Hs01573837_g1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells	NM_001165412.1	Hs00765730_m1
IL-1α	Interleukin 1 alpha	NM_000575.3	Hs99999028_m1
IL-1β	Interleukin 1 beta	NM_000576.2	Hs99999029_m1
IL-6	Interleukin 6	NM_000600.3	Hs99999032_m1
IL-8	Interleukin 8	NM_000584.3	Hs99999034_m1
IL-10	Interleukin 10	NM_000572.2	Hs99999035_m1
TNF-α	Tumor necrosis factor alpha	NM_000594.2	Hs99999043_m1
PRF1	Perforin 1	NM_001083116.1	Hs00169473_m1
iNOS	nitric oxide synthase 2, inducible	NM_000625.4	Hs01075529_m1
GZMA	Granzyme A	NM_006144.3	Hs00989184_m1
GZMB	Granzyme B	NM_004131.4	Hs01554355_m1
IL-15	Interleukin 15	NM_172175.2	Hs99999039_m1
MICA	MHC class I polypeptide-related sequence A	NM_001177519.1	Hs00792195_m1
MICB	MHC class I polypeptide-related sequence B	NM_001289160.1	Hs00792952_m1
KLRK1	killer cell lectin-like receptor subfamily K	NM_007360.3	Hs00183683_m1

Housekeeping genes selected to normalize expression levels			
Gene symbol	**Gene name**	**Accession No.**	**ABI Gene Expression Assay No.**
18S	18S ribosomal RNA	X03205.1	Hs99999901_s1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	NM_001289746.1	Hs99999905_m1
PGK1	phosphoglycerate kinase 1	NM_000291.3	Hs99999906_m1
GUSB	beta-glucuronidase	NM_000181.3	Hs99999908_m1
ACTB	beta-actin	NM_001101.3	Hs99999903_m1
B2M	beta-2-microglobulin	NM_004048.2	Hs99999907_m1

Supplementary Table E3: Clinical characteristics of control subjects included in the study. Sex: M, male; F, female. Symptoms: Py, pyrosis; Rf, reflux; D, diarrhea; AcR, acid regurgitation; AP, abdominal pain; Ht, heartburn; WL, weight loss. Endoscopy: N, normal; Atopy: BA, bronchial asthma; AR, allergic rhinitis; DS, drug sensitivity. ND, not determined.

Control subject	Age (years)	Sex	Reason for endoscopy	Endoscopy		Familiar background of atopy	Personal background of atopy
				Caliber	Mucosal appearance		
1	70	F	Py	N	N	ND	ND
2	63	M	Rf	N	N	ND	ND
3	30	M	D	N	N	ND	BA, AR
4	22	F	Ht	N	N	No	No
5	41	M	AcR	N	N	ND	ND
6	65	M	AP	N	N	No	No
7	80	M	D	N	N	ND	ND
8	61	M	AP	N	N	ND	AR
9	66	M	D	N	N	No	DS
10	32	M	D	N	N	No	No

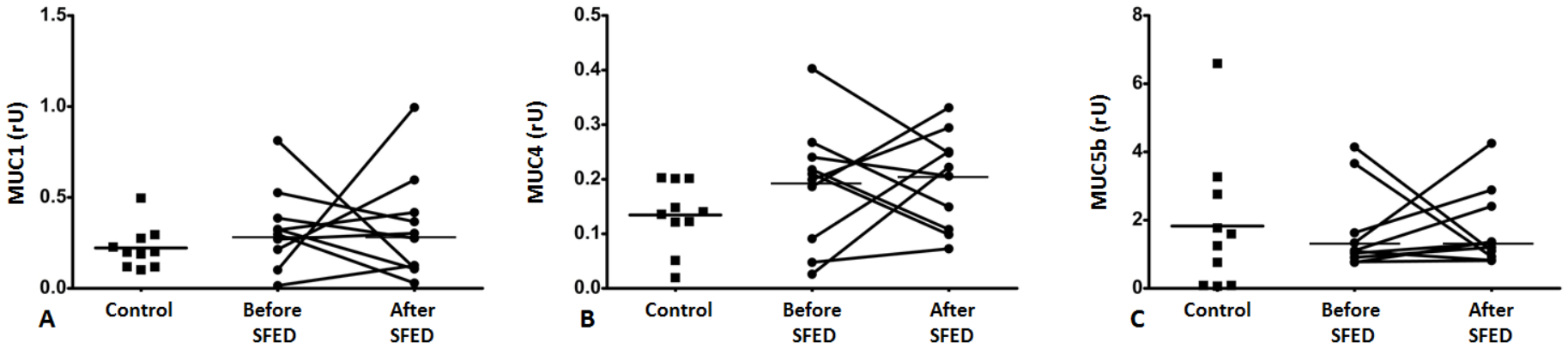

Figure E1: No changes in mucin gene expression in duodenal biopsies from EoE patients.

A-C) Mucins (Muc1, Muc4 and Muc5B) mRNA expression (in relative units) was determined in duodenal biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired t-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired t-test. Horizontal bars indicate mean values. (*p<0.05; **p<0.01; *** p<0.001).

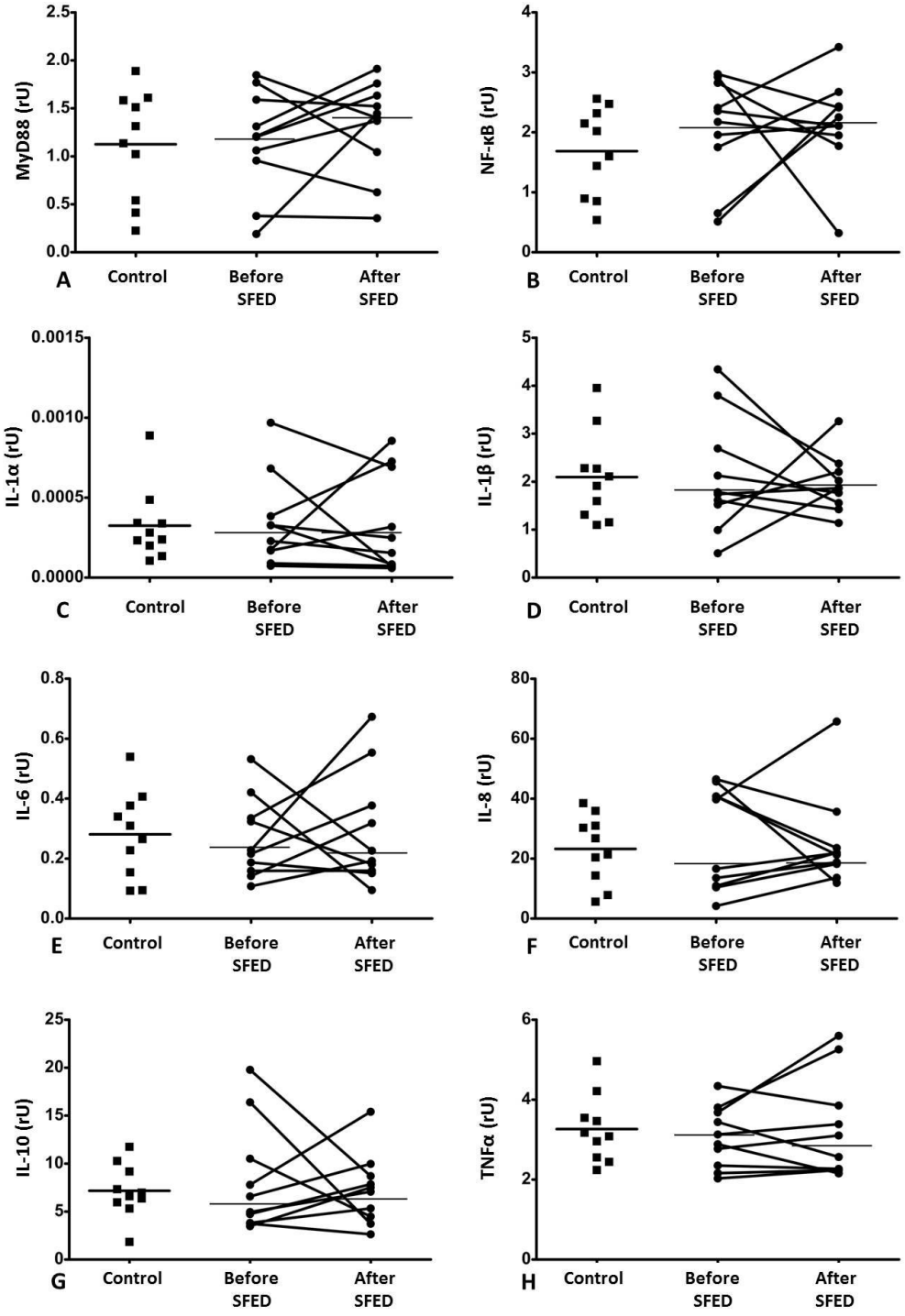

Figure E2: The TLR over-expression in the duodenum from EoE patients it not coupled with any further immune activation

A-B) Transcription factors (MyD88 and NF-κB) and **C-H)** cytokines (IL-1α, IL-1β, IL-6, IL-8, IL-10 andTNFα) mRNA expression (in relative units) was determined in duodenal biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired t-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired t-test. Horizontal bars indicate mean values. (*p<0.05; **p<0.01; *** p<0.001).

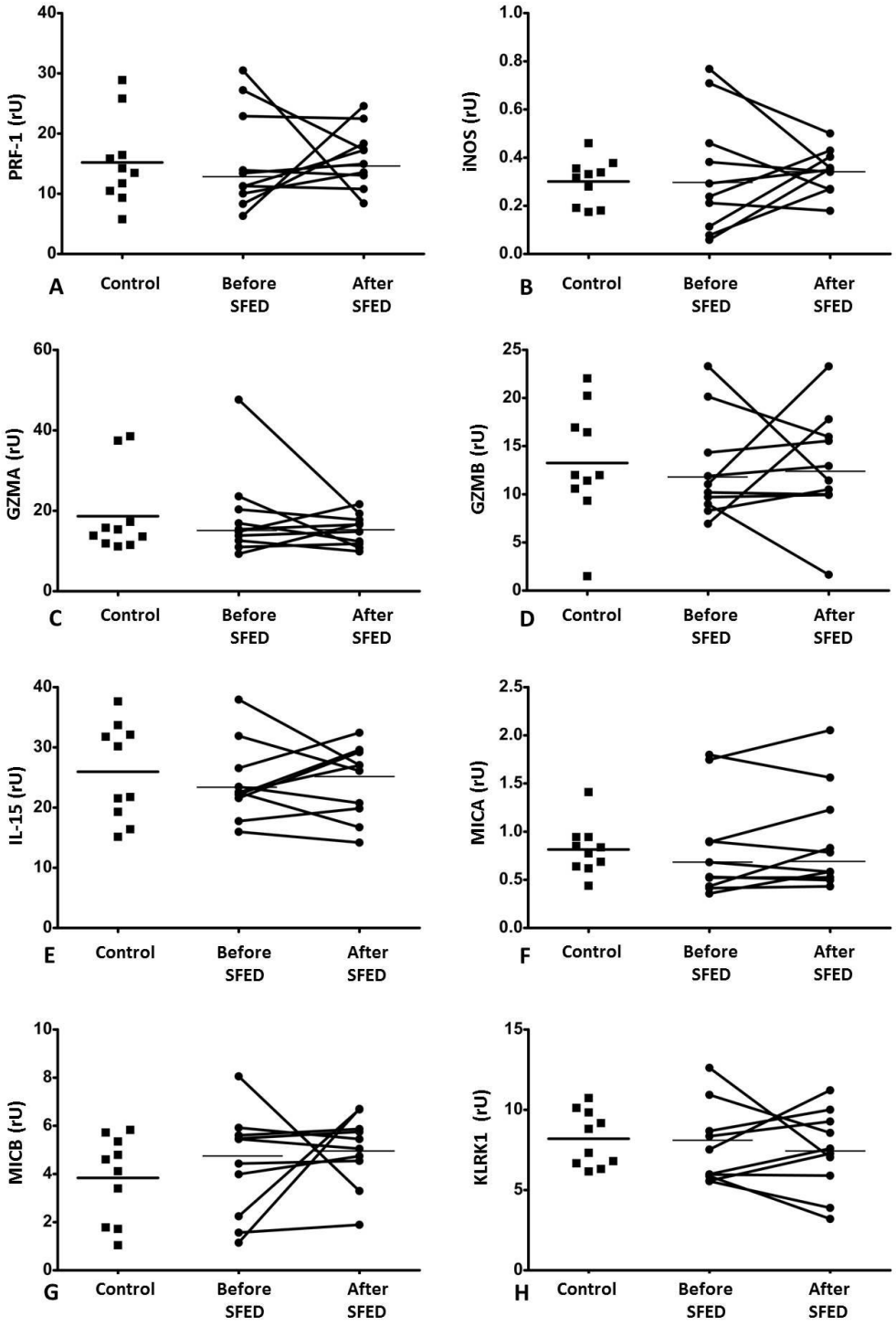

Figure E3: Absence of activation of the NKG2D system and changes in expression of effectors of inflammation in the duodenal mucosa of EoE patients.

A-D) Innate immunity effectors (PRF-1, iNOS, GZMA and GZMB); and **E-H)** the NKG2D system (IL-15, MIC-A, MIC-B and NKG2D) mRNA expression (in relative units) was determined in duodenal biopsies from patients before and after six-food elimination diet (SFED) treatment, and healthy controls. Paired t-test compared EoE patients before and after SFED, while EoE patients (both before and after SFED) were compared with the control population by non-paired t-test. Horizontal bars indicate mean values. (*p<0.05; **p<0.01; *** p<0.001).

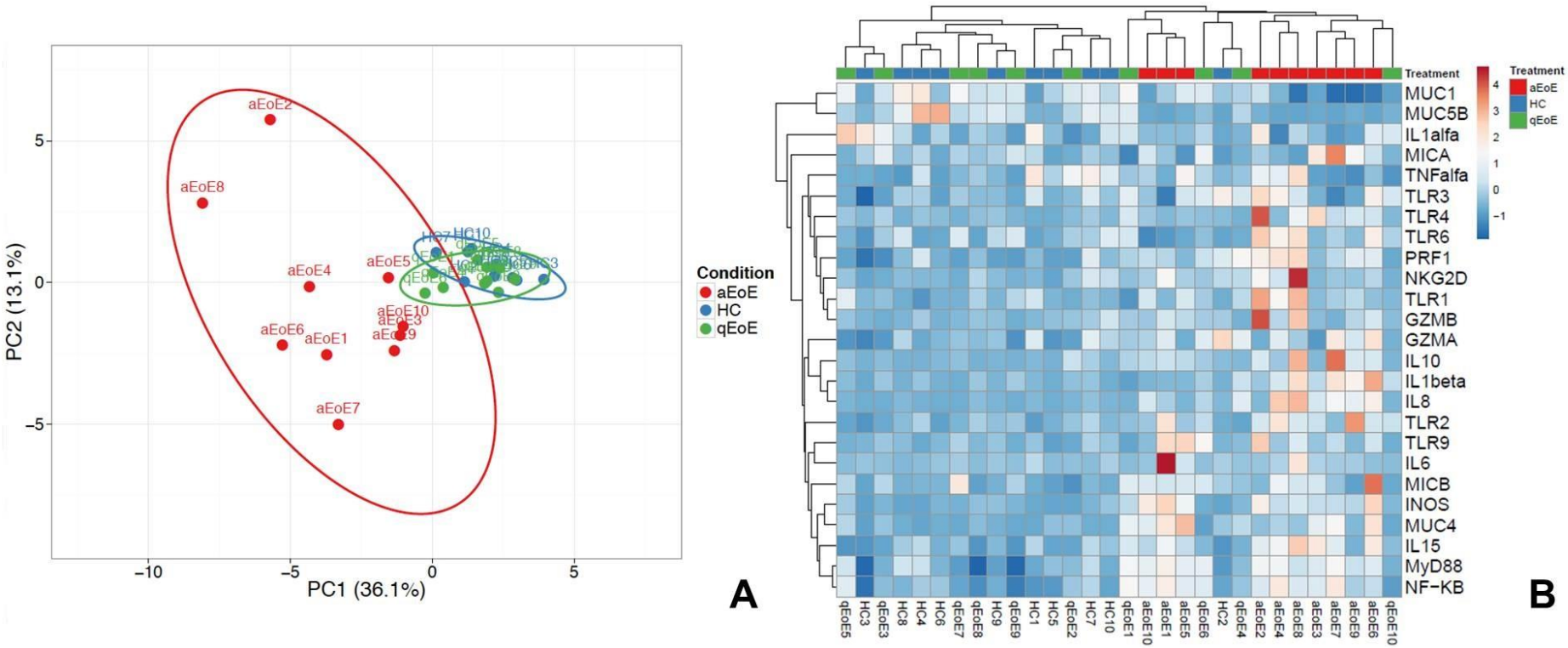

Figure E4: Genetic signature of the EoE esophagus

A) Principal component analysis (PCA) and **B)** Heatmaps were determined using all genes detailed on Table 2 from the duodenum of active (aEoE) and quiescent (qEoE) patients and healthy controls.

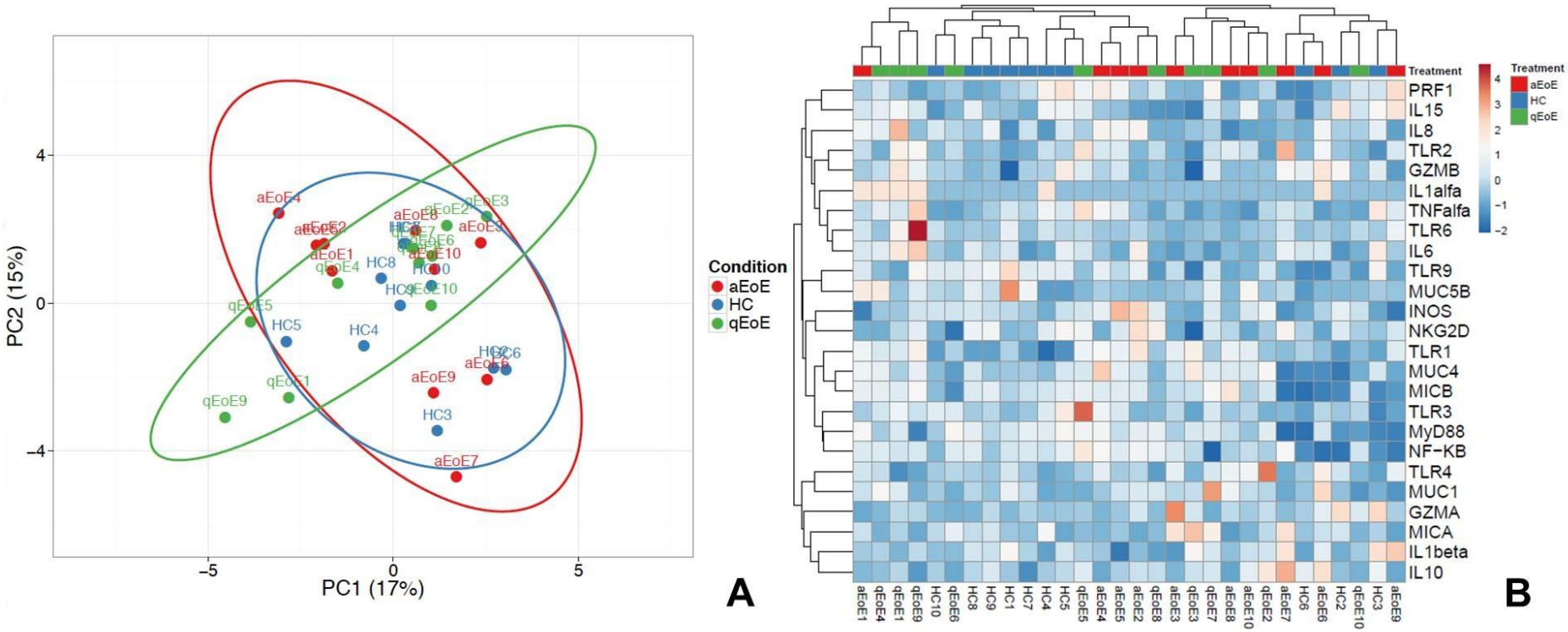

Figure E5: No differential gene expression profile in the EoE dudoenum

A) Principal component analysis (PCA) and **B)** Heatmaps were determined using all genes detailed on Table 2 from the duodenum of active (aEoE) and quiescent (qEoE) patients and healthy controls.

Artículo 4: Molecular basis and celular mechanisms of eosinophilic esophagitis for the clinical practice

Expert Rev Gastroenterol Hepatol. 2019 Feb;13(2):99-117
doi:10.1080/17474124.2019.1546120
***JCR Factor de Impacto: 2,963**;*
*Categoria: Gastroenterology & Hepatology 42/80 (**Q3**)*

EXPERT REVIEW OF GASTROENTEROLOGY & HEPATOLOGY
2019, VOL. 13, NO. 2, 99–117
https://doi.org/10.1080/17474124.2019.1546120

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REVIEW

Molecular basis and cellular mechanisms of eosinophilic esophagitis for the clinical practice

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ABSTRACT

Introduction: Eosinophilic esophagitis (EoE) is a chronic, allergen-driven inflammatory esophageal disease characterized by predominantly eosinophilic inflammation leading to esophageal dysfunction. Recent efforts to understand EoE have increased our knowledge of the disease.

Areas covered: Multiple cells, molecules, and genes interplay with early life environmental factors in the pathophysiology of EoE to converge in the esophageal epithelium at the center of disease pathogenesis. Epithelial cells constitute a mayor cytokine source for TSLP and Calpain-14; an impaired epithelial barrier function allowing penetration of food and microbiota-derived antigens is involved in triggering and maintaining inflammation. Eosinophil and mast cell-derived products, including TGFβ, together with IL-1β and TNFα, promote epithelial mesenchymal transition in EoE, contributing to tissue remodeling by synthetizing and depositing extracellular matrix in subepithelial layers. This article aims to provide a state-of-the-art update on the pathophysiology of EoE applied to clinical practice, and latest research and developments with potential interest to improve the diagnosis and treatment of patients with EoE are revised.

Expert commentary: Preliminary approaches have provided promising results toward incorporating minimally invasive methods for patient diagnosis and monitoring in clinical practice. Early diagnosis and optimized therapies will allow for personalized medicine in EoE.

ARTICLE HISTORY
Received 6 September 2018
Accepted 6 November 2018

KEYWORDS
Diagnosis; eotaxin-3; eosinophilic esophagitis; eosinophils; food allergy; interleukin-13; inflammation; immunopathogenesis; microbiota; mast cells; remodeling; therapy; thymic stromal lymphopoietin

1. Introduction

Eosinophilic esophagitis (EoE) is a chronic immune-mediated inflammatory disorder of the esophagus, defined symptomatically by esophageal dysfunction and histologically by eosinophil predominant inflammation restricted to this organ [1]. Initially characterized as a distinct clinicopathological disorder in the early 1990s [2,3], the incidence and prevalence of EoE have rapidly increased in children and adults in the last two decades to constitute a common cause of esophageal symptoms in clinical practice [4]. EoE is the most prevalent cause of chronic or recurrent esophageal symptoms after gastroesophageal reflux disease (GERD) and the main cause of dysphagia and food impaction in children, adolescents, and young adults in Europe and North America [5], where it affects 1-to-2 per 2000 inhabitants [6,7]. It is also emerging in other regions, including developing countries. As a result, EoE represents a large financial burden to the health care systems, with an estimated annual health-care cost of up to $1.4 billion in the United States [8]. The continually developing epidemiology of the disease, its chronic nature and the need to involve multidisciplinary teams in its management, demand the need for further research to understand the ultimate causes of the disease [9], to optimize the cost-effectiveness of the interventions, and finally, to plan preventive strategies.

Efforts to understand EoE have sharply increased in recent years, making it one of the topics of greatest interest among gastroenterologists and allergists. Research papers addressing the many aspects of EoE have increased almost exponentially as the disease is being recognized in multiple settings. In addition, pharmaceutical and biotechnological companies have acknowledged the unmet needs of EoE patients and are currently allocating resources to the potentially expanding market for EoE diagnosis and therapeutics. After 20 years of research on the causes of this disorder, large-scale epidemiological studies to define potential risk factors are still needed however. Integrating knowledge from genetic susceptibility loci proposed for EoE with environmental factors is required, and efforts should be made to develop non or minimally invasive tests for EoE diagnosis and monitoring. In addition, the optimal management of EoE patients remains controversial and widely variable [10–14] and treatment in clinical practice varies more than any other aspect related to the disease [11,13].

This article aims to provide a state-of-the-art update on the pathophysiology of EoE applied to clinical practice, and an updated review of the latest research and developments with potential interest to improve the diagnosis and treatment of patients with EoE.

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2. Esophageal eosinophils: from the cell to the histopathology

2.1. Eosinophils in the gastrointestinal tract and its trafficking to the esophagus

Eosinophils are granulocytes of myeloid lineage produced in the bone marrow and traditionally considered to be IgE-dependent effector cells that arise in inflammatory processes in response to allergic hypersensitivity and parasitosis. In normal conditions they are present in many tissues, including the mucosa of most segments of the gastrointestinal (GI) tract where they are extremely common, except in the esophagus, which is the only digestive organ that does not normally contain eosinophils. The ubiquity of these cells have led some authors to consider eosinophils to be regulatory cells involved in the maintenance of intestinal homeostasis [15] as opposed to the more conventionally active role they play in several intestinal diseases -including ulcerative colitis or EoE- and similar which occurs in bronchial asthma [16].

In order to achieve the high numbers of eosinophils that are detected in all layers of the esophagus in patients with EoE, they need to have first proliferated and matured in the bone marrow under the regulatory effect of several cytokines and growth factors. Among these Th2-cytokine interleukin (IL)-5 is the most specific and better studied for the selective expansion of eosinophils and their further release into the circulating blood [17] and was one of the first proposed therapeutic targets in EoE [18]. Research in murine models of the disease showed that transgenic mice with overproduction of IL-5 suffered from blood eosinophilia and intense eosinophils accumulation in the esophageal tissues, including the lamina propria, as well as in the small bowel after inhaled [19,20] or epicutaneous [21] stimulation with allergens, which was proportional to the serum concentration of IL-5 [16]. Deletion in the *IL-5* gene however protected mice from developing tissue eosinophilia after allergen stimulation [20]. The *IL-5* gene and its protein are upregulated in esophageal biopsies from active EoE patients [22,23]; the blood lymphocytes of EoE patients produce significantly higher levels of IL-5 following *in vitro* stimulation compared to normal controls [24] and the percentage of blood-circulating IL-5+CD4 T cells correlates with the severity of esophageal tissue eosinophilia [25]. Assessing the effectiveness of blocking IL-5 with monoclonal antibodies was, therefore, predictable.

Trafficking of eosinophils to the esophagus is accounted for the effect of several activation signals released from the inflamed tissue, which first induce the acquisition of tissue-specific functional properties in blood eosinophils. These differ not only depending on the tissue they exert inflammatory functions on (such as the esophageal, bronchial or colonic mucosa) [16], but also according to patients' age [26] and the disease status activity [25]. Despite the effect of homing molecules in the recruitment of eosinophils toward the esophageal mucosa having not yet been assessed, preliminary research, mainly with flow cytometry, has begun to delineate specific peculiarities of blood eosinophils which are able to lead them toward an inflamed esophagus: circulating blood eosinophils in EoE exhibit an enhanced expression of the CC chemokine receptor CCR3 common for eotaxins [25], the low-affinity receptor for IgE (CD23), the intercellular adhesion molecule (ICAM)-1 (or CD54) [16,26], integrin CD11c, the receptor for prostaglandin D2 CRTH2 [16,26] and FOXP3 mRNA [26]. Some of these have been assessed as potential therapeutic targets for EoE.

2.2. Therapeutic interventions for esophageal trafficking of eosinophils

The IL-5 blocker mepolizumab was tested in randomized controlled trials (RCT)s involving children [27] and adults [28], while reslizumab was evaluated in children only [29], neither of them demonstrating significant differences between the active and placebo groups in terms of symptom relief nor histological remission. Reslizumab has been suggested as being effective in children with EoE when used in the long term [30].

A selective CRTH2 antagonist (OC000459) with proven efficacy against eosinophilic asthma was assessed in a double-blind, placebo-controlled RCT in adult patients with EoE [31]: the drug induced a significant decrease in both esophageal eosinophilia and symptoms, with a trend toward improvement in endoscopic abnormalities compared with a placebo. However, esophageal mucosa did not revert to normal.

Selective, competitive antagonists of CCR3 are potentially promising drugs that are being investigated in bronchial asthma (an eosinophilic inflammation in the airways). As yet, no studies in EoE with these drugs have been proposed.

3. The epithelial cell: a central player in the pathophysiology of EoE

Epithelial cells are increasingly recognized as major components of the innate immune system that play a role in defensive functions of the GI mucosa [32]. The intestinal epithelium is crucial for preserving gut homeostasis and acts both as a physical barrier and as a coordinating hub for immune defense and crosstalk between bacteria and immune cells. If deregulated, the immunomodulatory function of epithelial cells may contribute to the development of intestinal inflammation. Cumulative research data are placing the esophageal epithelium in the center of the pathogenesis of EoE. As previously described with epithelial cells from various tissues including nasal, airway and intestinal mucosa [33,34], the esophageal mucosa is able to express major histocompatibility complex (MHC) class II molecules during inflammation [35,36] and thus behave as non-professional antigen-presenting cells [25,26].

The esophageal epithelium is a relatively impermeable surface unable to be passed through by medium and large-size molecules. It has also been demonstrated that superficial layers, but not basal and suprabasal ones, are those involved in establishing the esophageal epithelial barrier [37]. The eosinophilic infiltration in EoE is usually organized in a density gradient toward the more superficial layers and is more abundant on the strata in contact with the esophageal lumen (the contact point with swallowed allergens) [38]. In fact, eosinophils frequently cluster to form microabscesses within these superficial strata [39,40].

There is evidence that active EoE is characterized by an impaired barrier function caused by epithelial barrier defects [41], with reduced expression of E-cadherin, desmoglein-1,

involucrin and filaggrin, all being structural proteins involved in maintaining mucosal integrity. Tight junctions (TJ) are multiprotein junctional complexes that prevent leakage of transported solutes and water by sealing the paracellular pathway. The expression of some of their components (as claudin-1, claudin-4, claudin-7, occludin, and zonula occludin-1 proteins) has also shown alteration in patients with active EoE [42,44]. In addition, active eosinophilic inflammation alters the expression of the cytoskeletal protein synaptopodin in the esophageal epithelium [45].

Very recently, the origin of all these changes has been related with a depletion of the serine protease inhibitor, kazal type (SPINK) 7, a antiprotease, which is part of the differentiation program of the esophageal epithelium. SPINK7 was practically absent in esophageal biopsies taken from adults and children with active EoE but was prevalent in biopsies from healthy people. To demonstrate the role of SPINK7 in the pathophysiology of EoE, *SPINK7* expression was silenced in an esophageal epithelial cell line and primary esophageal epithelial cells, which lead to barrier dysfunction and transcriptional changes, characterized by loss of cellular differentiation and altered gene expression able to stimulate allergic responses with production of proinflammatory cytokines. Changes associated with SPINK7 silencing were reversed after treating the culture with antiserine protease α1-antitrypsin [46].

As a consequence of the above, an increased permeability has been demonstrated in patients with active EoE [47,48], which is translated at a tissue level by dilated intercellular spaces, an usual finding repeatedly reported in EoE patients of all ages [44,49]. This impaired barrier function might allow pathogens to invade the esophagus, and facilitate antigen penetration in active EoE patients. In fact, biopsy samples from active EoE are characterized by overexpression of epithelial antimicrobial peptides (mainly beta-defensins, cathelicidin LL-37, and psoriasin) [41] and upregulation of bacterial pattern recognition Toll-like receptors (TLR) [50]. Differences in anti-gliadin staining among patients with active and inactive EoE also suggest presence of intraepithelial food antigens in patients with active disease [51]. Both facts potentially contribute to perpetuate the inflammatory condition in EoE (Figure 1).

The esophageal epithelium is also the main source for thymic stromal lymphopoietin (TSLP), a cytokine with a central role in EoE. TSLP is mainly produced by non-hematopoietic cells such as epithelial cells, fibroblasts, and different types of stromal cells and its expression is linked to many allergic and immune-mediated diseases including asthma [52], atopic dermatitis [53], inflammatory bowel disease [54] and EoE. The factors inducing the release of TSLP are not clearly defined, but it plays an important role in the activation of antigen-presenting cells, including the food antigen-presenting dendritic cells in the esophageal mucosa, to promote maturation of T cell populations and inducing Th2 polarization of naïve CD4 + T cells [55,56]. These Th2 cells then secrete Th2 cytokines, including IL-13. IL-13 is a Th2-type cytokine with pleiotropic effects that play a key role in EoE. *IL-13* gene expression is upregulated in the blood eosinophils of patients with several eosinophilic inflammatory disorders including EoE [57] and especially in the esophageal epithelium of EoE patients compared with healthy controls [58]. The key role of IL-13 in the pathophysiology of EoE is supported by the fact that human esophageal cell cultures stimulated with IL-13 selectively induce the expression and secretion of the eosinophil-activating chemoattractants eotaxin-1/CCL11 and eotaxin-3/CCL26 [59], operating through the nuclear transcription factor STAT6 (which plays a central role in Th2 cell differentiation) [58], and are capable of partially reproducing the characteristic EoE transcriptome. This can then be reversed after topical steroid treatment in parallel with a significant reduction in *IL-13* mRNA expression levels [58]. In murine models, intratracheal delivery of IL-13 induces experimental EoE, whereas IL-13-deficient mice and those with a targeted deletion of STAT6 have attenuated degrees of allergen-induced experimental EoE and are partially protected from allergen- and IL-13-induced experimental EoE, respectively [21].

IL-13 promotes epithelial dysfunction in EoE: A decreased expression in filaggrin (*FLG*) and involucrin (*IVL*) genes is documented in IL-13-stimulated esophageal epithelial cells and that obtained from EoE patients compared with normal biopsy specimens [60]; IL-13 also reduces the adhesion molecule desmoglein-1 [61], inhibits the expression of filaggrin and involucrin, and alters the expression pattern of TJ-associated proteins [37]. The disruptive effects of IL-13 on the esophageal epithelium are regulated through the *CAPN14* gene, which is encoded in the EoE-susceptibility locus 2p23 and codified for Calpain-14 (CAPN14), an esophageal-specific protease with a role in protecting the integrity of esophageal tissue [62]. The *CAPN14* gene is dynamically upregulated by both IL-4 and IL-13 and exerts a gatekeeper role in EoE. Upregulation of CAPN14 is linked to impairment of the epithelial barrier, partially mediated by loss of DSG1, whereas its down regulation leads to failure in repairing IL-13–induced epithelial changes [63].

3.1. Epithelial products as diagnostic markers of EoE

The histologic method is the gold standard of an EoE diagnosis in patients with suggestive symptoms. However, EoE clinical symptoms do not always correlate with histology [64], and the patchy distribution of EoE limits the proper assessment of the disease if a minimum of 5 to 6 biopsies are not obtained [65]. EoE is characterized by a well-preserved genetic transcriptome, which was first discovered in 2006 [66], and led to the development of the EoE diagnostic panel (EDP), a novel molecular tool built on a Taqman®-qPCR-based low-density array system, which has the additional advantage of identifying histologically ambiguous subjects who may later develop active EoE [67]. By combining expression levels of 77 genes, the EDP identified adult and pediatric patients with EoE with approximately 96% sensitivity and 98% specificity, and distinguished patients with EoE in remission from controls, as well as identified patients exposed to swallowed glucocorticoids. A large prospective study validated the EDP, additionally demonstrating its feasibility from a single paraffin-embedded esophageal biopsy [68]. Among genes represented in the EDP, the epithelial-related ones were an essential component, with those codifying for filaggrin (*FLG*), Uroplakin-1a (*UPK1A*), serine peptidase inhibitor kazal-type (*SPINK*)7, cysteine-rich secretory protein (*CRISP*)3 and mucin (*MUC*)4 as the major representatives.

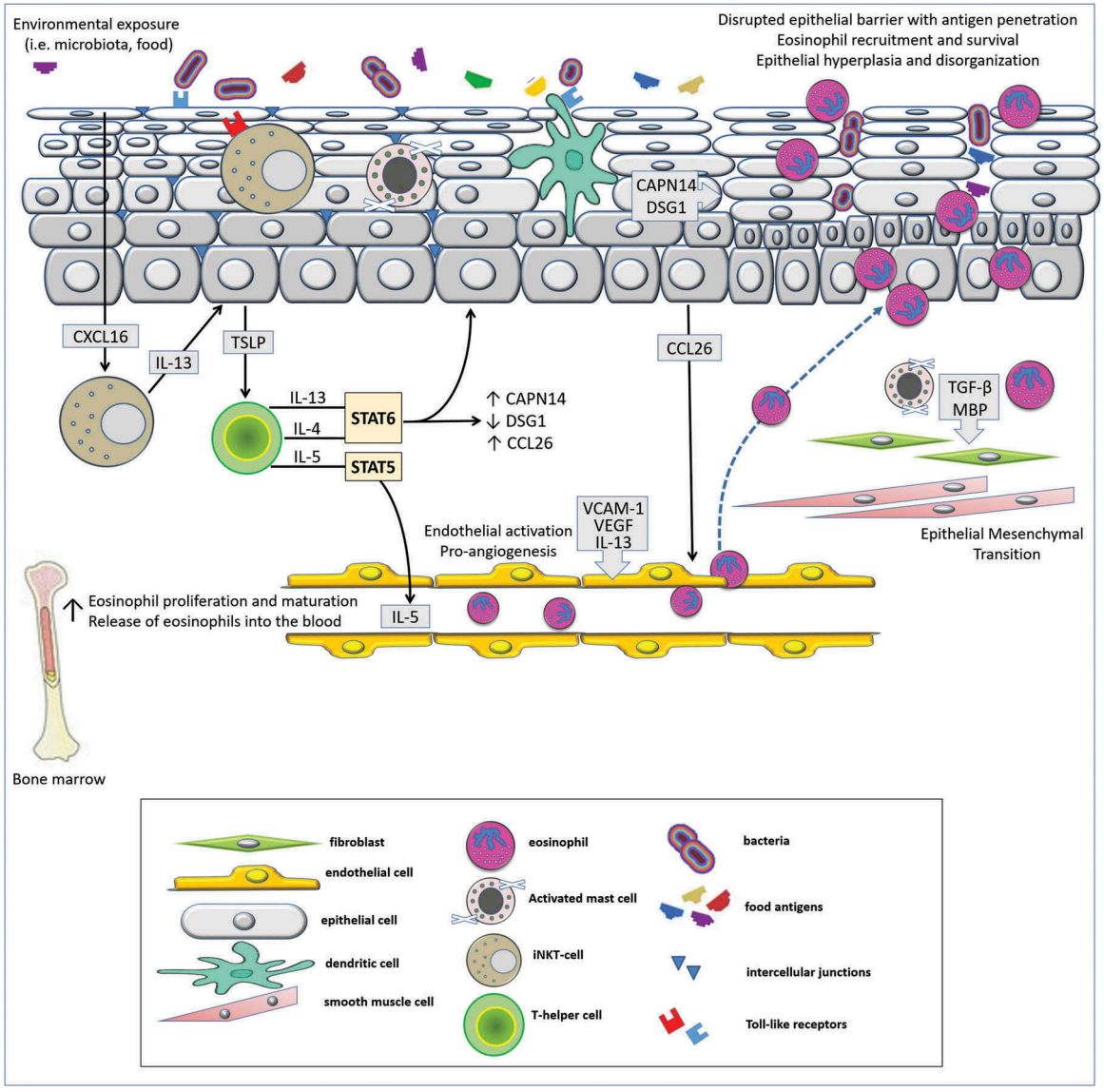

Figure 1. The esophageal epithelium in eosinophilic esophagitis as an immunologically active surface, which initiates and perpetuates inflammatory and structural changes characterizing eosinophilic esophagitis (EoE).

The activation of epithelial and dendritic cells after exposure (or lack of exposure) to components of the esophageal lumen (i.e., bacteria and food antigens) induce CXCL16 expression, which directly promotes invariant natural killer T (iNKT) cells recruitment. iNKT cells are the major source of Th2 cytokines, including IL-13, which directly induces changes in the gene expression pattern on epithelial cells, leading to thymic stromal lymphopoietin (TSLP) secretion. TSLP act on T-helper cells, promoting secretion of Th2 cytokines IL-13, IL4, and IL-5. IL-13, primarily acting together with IL-4 through signal transducer and activator of transcription 6 (STAT6) promotes the transcription of calpain-14 (CAPN14) and C-C Motif Chemokine Ligand 26 (CCL26 or eotaxin-3). While the first contributes to disrupt the epithelial surface which increases its permeability by decreased expression of the tight junction protein desmoglein 1 (DSG1) among others, CCL26 is a potent chemoattractant for eosinophils and mast cells. IL-5 also promotes tissue recruitment and survival of eosinophils signaling primarily through STAT5. Th2 cytokines also trigger the production of IgE by plasma cells. Activated eosinophils are multifunctional cells that regulate diverse processes including angiogenesis and endothelial activation by releasing vascular cell adhesion molecule 1 (VCAM-1), and vascular endothelial growth factor (VEGF) which are needed for recruiting inflammatory cells toward the esophagus. The effects of transforming growth factor 1 (TGF-1) and other activated eosinophil and mast cell-derived mediators on smooth muscle fibers (as major basic protein or MBP) lead to hyperplasia and hypercontractility. At the same time, they are key mediators for activation and proliferation of fibroblasts and for the subsequent synthesis of extracellular matrix components. Eosinophils themselves regulate the process of epithelial–mesenchymal transition, acting in a paracrine environment characterized by the presence of Th2 cytokines and eotaxins.

3.2. Therapeutic targets focused on epithelial function in EoE

Several studies have demonstrated that well-established therapies for EoE are able to restore the impaired esophageal barrier by improving epithelial integrity and reducing its permeability. This has been shown for elemental diet [69], proton pump inhibitors (PPIs) [70] and topic swallowed steroids [44,71]. The esophageal expression of gene encoding for several barrier integrity proteins -filaggrin, desmoglein-1, zonula occludin-3, and claudin-1, was impaired at baseline and restored after diet or steroids to similar levels to subjects with no esophageal disease [69,71,72]. This was manifested by normalization of esophageal impedance and transepithelial small molecule flux [69,72].

With regard to investigational products, anti-TSLP antibodies have been assessed in murine models of atopy, including asthma and EoE. TSLP antibodies or antibodies that inhibit its receptor TSLPR block CD4 Th2 development in asthma or allergic rhinitis in mice [73,74,75], and were shown to block the development of esophageal eosinophilia and food-related symptoms in experimental EoE [55]. As for human research, a fully human anti-TSLP monoclonal antibody that specifically

binds human TSLP (tezepelumab or AMG 157), preventing interaction with its receptor, has been tested in a phase IIb trial in adult patients with uncontrolled asthma with favorable effects [76]. TSLP is also a potent chemoattractant for eosinophils, thus reinforcing the activity of this drug [77], making this product a promising pharmacological target also for EoE [78].

Anti-IL-13 antibodies have also been assessed in EoE patients in clinical trials. The first one investigated QAX576 as a potential treatment of adult EoE and was published in 2015. Patients were randomly assigned to QAX576 (6 mg/kg) or placebo every 28 days for 3 doses with 6-month follow-up. QAX576 led to a decrease in mean intraepithelial eosinophil counts but reached no histologic remission, and a non-significant trend toward improvement in dysphagia severity, as measured by the Mayo Dysphagia Questionnaire, was documented. In addition, QAX576 normalized the expression levels of some EoE-related genes, including *eotaxin-3/CCL26, periostin* (*POSTN), carboxypeptidase A3* (*CPA3*), and *desmoglein-1* (*DSG1*). Transcriptional changes differed between responders and nonresponders to QAX576 [79].

IL-13 exhibits a 30% sequence similarity with IL-4 and both share similar structures. *IL-13* (but not *IL-4*) gene expression is upregulated in the esophageal epithelium of EoE patients compared with healthy controls [58]. However, both cytokines mediate downstream effects via a common heterodimeric receptor, IL-4Ra and IL-13Ra1. It has been proposed that therapies targeting IL-4 and IL-13 separately may be ineffective because IL-4 and IL-13 have overlapping downstream effects [80]. Dupilumab, a monoclonal antibody against IL-4Ra, is the most promising IL-4/IL-13-targeted therapy to date. After demonstrating effectiveness in asthma [81] and atopic dermatitis [82], ongoing trials are now assessing dupilumab in EoE [83]. A phase II, randomized, double-blind, placebo-controlled clinical trial (NCT02379052) was carried out with 47 participants to assess the clinical efficacy of a 12-week treatment period with dupilumab for relieving symptoms in adult patients with active, moderate-to-severe EoE [84,85]. Patients received either dupilumab 300 mg weekly following a 600-mg loading dose or placebo. At week 10, patients who received dupilumab reported a significant improvement in the ability to swallow compare to placebo (45% *vs*. 19% improvement from baseline in the Straumann's Dysphagia Symptoms Score). Esophageal eosinophil counts significantly reduced by 93% from baseline in patients who received dupilumab weekly compared with an increase of 14% in those who received placebo. Long term assessment of the effectiveness of dupilumab in the sustained control of EoE is required.

4. EoE as an allergic disease

EoE constitutes a particular allergic condition triggered and maintained by food allergens [86–88], with a potential role for aeroallergen exposure in the genesis and exacerbations of EoE which is not supported by most of the current evidence [87,88]. Atopy has been linked to EoE since the initial reports of the disease, with most patients having a family history of bronchial asthma or allergic rhinitis; atopic dermatitis; hypersensitivity to drugs, blood eosinophilia; or elevated serum total and specific IgE levels [89]. IgE-mediated food allergy is also common among EoE patients and alters its clinical presentation during childhood [90]. Overall, atopic manifestations are 3 to 5 times more common among patients with EoE compared with control subjects [91]. The definitive demonstration of EoE as a food allergy came in 1995, when Kelly *et al*. provided firm evidence of resolution of histological lesions and symptoms in pediatric patients following elemental amino acid-based diets lacking antigenic capacity [92]. In children, retrospective cohort analyses have suggested that EoE is a late manifestation of the allergic march in some individuals, with a peak of incidence which appears after that of atopic dermatitis, IgE-mediated food allergy and bronchial asthma. There was also a cumulative effect of multiple preceding allergic conditions in the rate of subsequent EoE diagnosis, which was higher in individuals with more than one preceding allergic condition [93].

4.1. iNKT lymphocyte responses as initiators of allergy and EoE

Inflammatory responses in food allergy, including EoE and atopic dermatitis, occurs on the epidermal border and are closely related to the microbiota and its metabolites able to modulate host immune responses [94], leading to the development of both tolerance or allergy. The global increase in all kind of allergies and immune-mediated diseases, especially in industrialized countries to represent a major health concern has been interrelated through the hygienic hypothesis. That is, reduced exposure to microorganisms during childhood has modified the patterns of gut microflora, leading to a change in the fine tuning of Th1, Th2 and regulatory T-lymphocytes (Treg) responses [95]. A lack of appropriate immune stimulation during early childhood leads to disturbed alignment in the sequence of encountering self- or non-self-antigens and accounts for the rise of atopy and autoimmune disease. A central role of 'training' regulatory T-cells through sufficient microbial exposure, leading to a robust, healthy balance between inflammation and anti-inflammation or immune tolerance has been recognized in the so call 'early immune challenge hypothesis' [96].

Invariant natural killer T (iNKT) cells are innate-like T cells that recognize glycolipid antigens rather than protein antigens via the MHC class I-like protein, CD1d, which is involved in the initial phases of a great variety of immune responses from oral tolerance to autoimmunity [97]. iNKT cells rapidly produce Th2-type cytokines (IL4, IL5 and IL13), as well as eotaxins; this leads to IgE production and subsequent sensitization to protein antigens [98]. Thus, iNKT cells play an important role in affecting the pathogenesis of allergic diseases. An age-sensitive contact with commensal microbes is critical for establishing mucosal iNKT cell tolerance to later environmental exposures [99,100]. When early-life microbial signals are not provided to mucosal tissues that are usually exposed to commensal microbiota, such as the intestine and airways (either by restricting microbial exposition or by using antibiotics during the first year of life [101], an excessive and persistent accumulation of iNKT cells occurs [99]. Consequently, these mucosal tissues are rendered more susceptible to later-

life environmental triggers of iNKT cells, which will mediate allergic sensitization and tissue inflammation [102].

iNKT lymphocytes are recognized as the major source for pro inflammatory cytokines in EoE [103,104]. Thus, although iNKT cells primarily recognize glycolipid structures located in pathogenic bacteria [105,106] and presented by CD1d, they can also be activated by sphingolipids found in food. For example, milk sphingolipids were shown to activate peripheral blood iNKTs in EoE-active children, producing Th2 cytokines [104]. Sphingolipids are present in many other common foods, with the foods richest in these components (i.e., milk and eggs) being the major common triggers of food allergies and EoE [86] (Figure 1).

The contribution of iNKT cells to the pathophysiology of EoE have been recently demonstrated: In animal models, activation of iNKT is sufficient to induce EoE, while neutralization of iNKT cells protects against experimental EoE [107,108]. CD1-deficient mice are protected from experimental EoE [109]. EoE patients have reduced peripheral blood iNKTs, and increased esophageal iNKTs compared to controls. Additionally, iNKTs from patients with active EoE expand more readily and produce more IL-13 in response to stimulation when compared to controls [104]. A study on children with EoE provided compelling evidence of insufficient immune imprinting by environmental microorganisms resulting in esophageal upregulation of epithelial and dendritic cell-derived CXCL16 [103], a chemokine that induces chemotaxis of iNKT cells into the esophagus. Esophageal samples from children with EoE show an increase in iNKT cells and components that regulate its chemotaxis and activity. iNKT cells activity was more pronounced in patients with early-onset EoE, who also had high levels of sensitization to food allergens. The elimination of allergens from the diet normalized cellular markers of iNKT activity. The modulation of the CXCL16–iNKT–CD1d axis remains a challenging therapeutic target to be investigated not only for allergic disorders such as EoE, but also in inflammatory bowel disease, celiac disease and cancer therapy.

4.2. Immunoglobulin involvement in EoE

The generation of antigen-specific IgE induced by a Th2 cell-mediated class switching of plasma cells is a central process to the pathophysiology of multiple allergic disorders. The effect IgE over FcRI receptors to induce degranulation from mast cells and basophils leads to immediate responses, anaphylaxis representing the clearest and most severe example [110]. Total and food-specific serum IgE levels are usually increased in patients with EoE, who frequently show allergen-specific skin prick test (SPT) responses [111], providing evidence of an immediate hypersensitivity in EoE. B cells have been identified within the inflammatory infiltrate of EoE [38,112], which have been shown to perform class switching and generation of IgE locally within the esophagus of both atopic and non-atopic patients with EoE [112], similarly to shown in IgE-mediated conditions as bronchial asthma [113] and allergic rhinitis [114]. The esophageal lining acquires the characteristic elements of an IgE-mediated response such as dendritic cells [38,115], class-switched B-cells [112], tryptase-positive mast cells [55] and Th2-cytokines [23,58]. However, the role of IgE in EoE is not still clear.

Evidence points to the independent evolution of EoE and concurrent atopies in the same patients. The elimination of foods that give positive results on skin prick tests usually fails to achieve disease remission [116,117] even though positive skin prick testing (SPT) results are observed in more than 80% of adult patients [89]. Atopic features and allergy sensitization patterns in EoE appear to be no different from those in atopic individuals without EoE living in the same geographic area and exposed to common allergens [118] with no significant differences regarding history of allergic rhinitis, atopic dermatitis, IgE-mediated food allergy, sensitization to aeroallergens, and family history of atopy [119]. Demographic, clinical, and histopathologic esophageal features were identical in patients with EoE who did not present with other atopic manifestations. Serum levels of allergen-specific IgE and the results from SPT correlate poorly with the food trigger(s), with the response to food elimination diets being equally effective in patients with EoE with negative allergy test results [120]. Food reintroduction in EoE does not determine immediate responses such as anaphylaxis. IgE-deficient [55], and B-cell deficient [109] mice are able to develop experimental EoE, as well as those exposed to the IgE-independent aeroantigen Aspergillus [19], which supports the dispensability of IgE in the pathogenesis of EoE. Collectively, these observations suggest there are other non-IgE-mediated pathways important in the EoE pathogenesis. Common genetic and environmental etiologic factors that contribute to the independent development of atopy and EoE might explain the association of both entities [101,121].

Recently, an increasing role for IgG4 in EoE is been recognized, after a seminal study which demonstrated a 45-fold increase in IgG4 concentration compared to controls in the esophageal tissues of adult EoE patients with active disease, as well as increased food-specific serum IgG4 to the foods that are most associated with EoE: milk, wheat, egg and nuts [122]. Additional studies in children [123–125] and adults [126] confirmed these results; tissue IgG4 levels correlated with esophageal peak eosinophil count, degree of histological features, *IL-4, IL-10* and *IL-13* gene expression level in subjects with EoE [125], thus supporting the potential role of IgG4 in EoE.

IgE and IgG4 are the most prominent isotypes of Ig in human immune responses to allergens. Similarities in allergen specificity patterns of IgE and IgG4 are due to their common dependence on IL-4 as a switching factor [127]. Upon natural exposure, IgE antibodies appear earlier, but exposure to most if not all allergens will induce substantial amounts of IgG4 antibodies [128]. Only upon frequent exposure the plasma IgG4 level rises and IgG4 becomes the dominating antibody [129], suggesting that IgG4 antibodies are associated with prolonged exposure to antigens, including food antigens. As a result, IgG4 has been involved in allergen-specific immunotherapy (AIT) in the treatment of IgE-mediated food allergy. In AIT, incrementally increasing doses of inciting allergen are given with the aim of increasing tolerance, initially through desensitization, which relies on regular exposure to allergen. With prolonged therapy in some subjects, AIT may induce sustained unresponsiveness, in which tolerance is retained after a period of allergen

avoidance [130]. Due to its poor capacity to activate effector cells or complement, IgG4 has been commonly associated with 'tolerance' and its appearance during the treatment of food allergy through oral immunotherapy (OIT) for food (one the methods of AIT) has been related to the protective role played by IgG4 in avoiding IgE-mediated responses after exposure to culprit antigens. Interestingly, together with enabling the production of IgG4, OIT is known to induce *de novo* EoE after being used to treat food specific IgE-mediated food allergy in up to 4% of patients [131]. The reasons why Ig4 seems to lose its tolerogenic capacity in these circumstances have not been clarified, but it has been proposed that T cells that home toward the esophagus in EoE enhance IgG4 antibody local production [128] or the role of eosinophils to support plasma cell survival [132] maybe also relevant in this condition.

4.3. Steroid treatment for EoE patients

As in other atopic disorders, topical steroids currently constitute the prevailing therapeutic option for EoE; the development of new formulations targeted to provide an optimal esophageal coverage suppose that they will probably continue to do so in the near future. Several RCTs summarized in sequential meta-analyses [133–136] have demonstrated that topically administered fluticasone propionate and budesonide are highly effective in children and adults, significantly superior to a placebo and comparable to oral prednisone [137] in inducing histological and symptomatic disease remission. However, despite the efficacy of steroids in treating the symptoms of EoE, their action is not sustained after discontinuation of medication. The ability of topical steroids to reverse EoE has been repeatedly demonstrated at a gene expression and molecular level [58,138], exerting their actions through a variety of mechanisms including transcriptional inhibition of specific promoter response elements, destabilization of cytokine mRNA and direct induction of cellular apoptosis. In the specific case of EoE, swallowed steroid therapy has been demonstrated to act topically and mediates its effects by directly regulating gene expression in esophageal epithelial cells [139]; thus, after binding to the glucocorticosteroid receptor, steroids repress IL-13-induced eotaxin-3 expression while induce FK506-binding protein 5 (*FKBP51*) gene expression. This inhibits glucocorticoid receptor-mediated signaling, which in turn represses IL-13-induced *eotaxin-3* promoter activity [139].

4.4. The anti-inflammatory effects of PPIs in EoE

The consideration of proton pump inhibitor (PPI) therapy within the diagnostic and/or therapeutic algorithm has been the most evolving topic in EoE over the past decade. As for patients with PPI-responsive esophageal eosinophilia (PPI-REE) , it was demonstrated that baseline expression of markers of Th2-mediated and eosinophilic inflammation (including *CCL26, IL-13, TSLP* and *POSTN*) in esophageal tissue largely overlaps in non-responders and responders to PPI therapy [22,70]. Patients with PPI-REE also showed a transcriptome that almost completely overlapped with non-responders to PPIs, including the hallmark EoE gene for eosinophil chemotaxis (*CCL26*), barrier molecules (*DSG1*), tissue remodeling (*POSTN*), and mast cells (*CPA3*) [140,141], constituting a genetic profile that was radically different from that observed in patients with GERD and control subjects. PPI monotherapy in PPI-REE patients can almost completely reverse the Th2 signature and normalize the EoE diagnostic panel expression [22,140], similar to other anti-inflammatory drugs, like topical steroids or anti IL-13 blockers. The molecular mechanisms whereby PPIs blocks Th2 cytokine-driven esophageal eosinophilia *in vitro*, independently of effects on gastric acid secretion, include its ability to inhibit IL-4 and IL-13-stimulated eotaxin-3 expression in esophageal cells and block STAT6 by binding the promoter [142,143].

5. Mast cells and other components of the inflammatory infiltration in EoE

Mast cells are mesenchymal bone marrow-derived myeloid cells widely distributed in vascular connective tissues. As a part of the innate immunity, they act against parasites and bacteria. In humans, mast cells are classified into two types depending on their granule content [144,145]: MC_T (mast cells with tryptase) and MC_{TC} (mast cells with tryptase and chymase). The mast cell population within the esophageal epithelium predominantly consists of MC_{TC} cells, both under normal conditions and in EoE [146]. This phenotypic diversity is not only a descriptor of tissue location, but also of the regulation of cytokine gene expression and, as such, is associated with functional differences [147–149].

A role for mast cells in the pathogenesis of EoE was proposed after studies demonstrated both their activation [150] and increased density in the esophageal mucosa of experimental [150,151] and human EoE in adults [23,38,146,152] and children [66,149,153–156]. These increases were significant compared with healthy controls as well as with patients with GERD; in fact, mast cell density has been proposed as a marker to distinguish GERD from EoE [153,157]. Several pieces of research have supported the potential role played by mast cells in EoE: Its density correlates with eosinophilic infiltration within the esophageal epithelium [158], with a reduction in both cell types after treatment with topical steroids [159,160] or anti-IL-5 [161], anti-IL-13 [79] or 6-food elimination diet [146], in association with clinical remission [146,152,160,162].

Mast cell infiltration, together with eosinophils, is directly associated and significantly correlated with symptoms in adult patients with EoE [146]: The peak number and activation of mast cells, and the expression of major mast cell proteases (including *CPA3, chymase/CMA* and *tryptase/TPSB2*) in the esophageal mucosa directly and significantly correlated with symptom scores in adult patients with EoE. Mast cell-mediators have been shown to be upregulated in EoE in several reports.

The expression of specific mast cell-mediators has also been shown to be upregulated in several reports [66,149,155], with mast cell-derived TGF-b1 contributing to esophageal dysmotility in both human [155] and murine experimental EoE [150] through

the induction of smooth muscle hypertrophy and hyperplasia, thus contributing to esophageal symptoms.

5.1. Activation of mast cells in EoE

Antigen cross-linking of IgE antibodies on the mast cell surface is the most extensively studied mechanism leading to mast cell activation and degranulation. This results in a rapid release of autacoid mediators and a sustained synthesis and release of cytokines, chemokines and growth factors [163] and leads to anaphylaxis as its most characteristic consequence. However, immediate systemic reactions to the foods responsible for EoE are not described in these patients, despite the fact that local IgE production has been demonstrated in the esophageal mucosa of patients with EoE regardless of their atopic background [112]. No differences in esophageal mast cell densities were shown between EoE patient with and without an atopic background [146], despite IgE-bearing mast cells being described in the esophageal epithelium of the former [164,165]. This suggests that IgE is not the main trigger of mast cell activation in EoE, with other IgE-independent mechanisms playing the principal roles. In fact, MC_{TC} are also strong responders to non-IgE -mediated regulation including the activation of toll-like receptors [166], exposure to gastric reflux [167,168], bile acids [169], the enteric nervous system [170], and certain eosinophil-derived proteins, mainly major basic protein [171]. In any case, the definitive exclusion of a putative role for IgE-promoting, mast cell-dependent, immediate reactions would require evidence of mast cell activation just after challenging a patient with a known food trigger for EoE, and this has yet to be demonstrated.

5.2. Treatments acting on mast cell activation in EoE

Cromolyn, as a mast cell stabilizer, is a first-line agent to treat GI symptoms of systemic mastocytosis with a poor absorption and almost nonexistent side effects. When used in patients with asthma it is able to significantly decrease activated eosinophils in bronchial mucosa, similarly to fluticasone propionate and superiorly to placebo or beta-2 agonists [172,173]. Early case reports in children with EoE failed to demonstrate a beneficial effect for cromolyn on symptoms and inflammation [174]. A very recent randomized placebo-controlled trial has structurally assessed viscous oral cromolyn for EoE in 16 pediatric patients [175]. Esophageal peak eosinophil counts and blood eosinophilia did not change after an 8-week treatment. A non-significant trend to symptoms improvement was documented in the intervention arm. It should be noted that MC_{TC} cells do not specifically respond to mast cell-stabilizer drugs such as cromolyn in the same way as MC_T cells, which are predominant in the bronchial mucosa and alveolar wall, a finding which explains the documented lack of efficacy of these drugs in treating EoE.

Montelukast, a leukotriene D4 receptor antagonist, is used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast also inhibits mast cell degranulation in the skin [176] and gastrointestinal tract mucosa [177] and has been assessed as a potential therapy for EoE. Used at standard doses in children [178] led to some symptomatic improvement in an open-label trial, with no patients achieving histologic response. Montelukast did not demonstrate superiority over placebo in maintaining remission in adult patients with EoE [179,180].

Omalizumab is an anti-IgE monoclonal antibody effective in controlling asthma in severely allergic asthmatic patients. It has also been assessed as a treatment for EoE in short case pediatric series [181] and recently in an open-label trial on 15 adolescents and young adults [182]. After 12 weeks, histological and clinical remission of EoE was documented in one third of participants, who were those with low peripheral blood absolute eosinophil counts. Finally, 30 adults with EoE were randomly assigned to receive omalizumab or placebo in a double-blind trial in which omalizumab did not alter esophageal symptoms or eosinophil counts in biopsy samples compared with placebo [122]. Despite these disappointing results, this trial observed granular deposits of IgG4, abundant IgG4-containing plasma cells, and serum levels of IgG4 reactive to specific foods in patients with EoE, indicating that, in adults, eosinophilic esophagitis is IgG4-associated, and not an IgE-induced allergy. Similar findings have been recently reproduced in children [123].

6. Genes and environment in EoE

As in other immunoallergic diseases, EoE arises from the interaction of environmental, host immunologic and genetic components [183,184]. The relative weight of each one in the final result of the disease has just begun to be defined. The contribution of genetic heritability to EoE has been defined in two family-based studies. In the first one, concordances for EoE among nonrelated individuals, siblings, dizygotic twins, and monozygotic twins were assessed. While the prevalence of EoE in the general population (or its general risk) was estimated in about 0.05% (1/2,000 inhabitants), it increased to 2,4% in siblings, 22% in dizygotic twins and 41% in monozygotic twins, despite the last sharing 100% of their genetic identity [183]. Since dizygotic twins and siblings have the same genetic relatedness, the authors used this difference to determine that environmental factors contributed 81% toward the phenotypic variance in the development of EoE. The contribution of genetic risk variants accounted for only 15% of the phenotypic variation of disease risk. More recently, a population-based genealogy resource linked to electronic medical records for health care systems across the state of Utah was used to estimate familial aggregation and risk of EoE in extended relatives to clarify the contribution of genetic factors to the disease [184]. Risks of EoE increased among first-degree relatives (OR 7.19), especially if they were diagnosed <18 years of age (OR, 16.3). In second-degree relatives and first cousins, the risk was also significant (OR 1.99 and 1.03, respectively). However, spouses of EoE patients were observed to be also at increased risk of EoE (OR 2.86), which suggested a shared environmental exposition leading to the disease.

To identify genes providing susceptibility to EoE, candidate gene approaches and genome-wide association studies (GWAS) were developed [185]. Microarray analysis of RNA

expression (or transcriptome) in EoE patients compared with control subjects shows significant changes in 1% of the human genome, which are remarkably conserved across sex, age and allergic status [66]. Eotaxin-3/*CCL26* is by far the most highly expressed gene in the EoE transcriptome, with a 53-fold increase compared with the controls. Both the TSLP receptor and its ligand seem to be implicated in the genetic links in EoE, especially after 5q22 (which contains the TSLP gene) was identified as a susceptibility locus for pediatric EoE through genome-wide association studies [186]. Single nucleotide polymorphisms (SNP) in *CCL26, TGFβ* and its binding protein LRRC32, *FLG, TSLP, DSG1, CRLF2* and *TLR3* genes have been described as risk factors for EoE [56,61,185–189]. The male predominance (~70%) traditionally described in EoE [4], implying that currently unidentified sexual chromosome-related genes or hormonal factors may be involved in the development of the disease, have been explained by a mutation in the X chromosome affecting two chains for the IL-13 receptor (IL-13 Ra 1 and 2 located in position Xq13.1–q28), which would remain uncorrected by the Y chromosome genes in males [189]. More recently, an SNP in the gene encoding for the TSLP receptor (*TSLPR*) located in the pseudoautosomal region on Xp22.3 and Yp11.3 has been shown to be directly involved in the male predominance of EoE [184]. The comorbidity of EoE with other allergic diseases and the involvement of some of the genetic variants in other diseases have given rise to the identification of specific EoE risk and esophageal tissue-related loci by GWAS, which was significant, independent of the sensitization status of the patients [190]. Among them *CAPN14* (located in 2p23), *TSLP* and *WDR36* (the second coding for a protein involved in facilitating multiprotein complexes) (located in 5q22), *LRRC32* and *C11orf30* (11q13) and the downstream primary mediator for IL-13 and IL-14 signaling *STAT6* (12q13) were the most relevant. However, the extent of the association with disease susceptibility for the currently described gene variants is modest (<2 fold), similar to the magnitude described in other allergic and immunologic diseases.

The potential role of environmental exposure in the etiology of EoE has been assessed in retrospective cohort studies and case-control designs. Despite appropriate inference, the overall risk of bias of these studies was high, with selection of patients being limited to single centers for the most part [191,192]. Available research showed that prenatal and early life factors seems essential to determine risk of EoE, including exposure to antibiotics during childhood [101,193,194], cesarean delivery [101,192–194], maternal fever, and preterm labor [192]. All these factors have been associated with dysbiosis in gut colonization in early life [195,196]. In contrast, having a furry pet in infancy has been proposed as providing a protective role [192]. Population density (rural versus urban) [197,198], aeroallergen exposition [116,199] and pollen season were also described as risk factors. For the later, a systematic review with meta-regression found no significant variations in the seasonal distribution of either the diagnosis or clinical recrudescence of EoE throughout the year [87]. A supposed inverse relationship between EoE and *Helicobacter pylori* infection [200,201] has been also excluded by a recent large case-control study [202].

The interplay between genes and environmental factors in EoE has only been assessed very recently in a preliminary study. Interactions between EoE-predisposing polymorphisms (within *TSLP, LOC283710/KLF13, CAPN14, CCL26*, and *TGFβ*) and early-life factors (antibiotic use in infancy, cesarean delivery, breast-feeding, neonatal intensive care unit admission, and absence of pets in the home) were tested in a case-control study recently published [203]. Interactions between rs6736278 (*CAPN14*) and breast-feeding ($p = 0.02$) and rs17815905 (*LOC283710/KLF13*) and neonatal intensive care unit admission ($p = 0.02$) were demonstrated, but not with the remaining factors examined. In addition, the authors found that breast-feeding had a strong protective effect in those with the susceptibility genotype in *CAPN14* gene, suggesting for the first time in the literature that risk of EoE disease might be modifiable in subjects with certain environmental exposures and gene variants.

Taken together, the evidence supports that EoE is a multifactorial and genetically complex disease, which involves an interplay between genetic predisposition and environmental factors, among which early life exposure likely to affect esophageal/gut microbiome content and diversity appear to be the most relevant.

7. Fibrous remodeling in EoE patients

Subepithelial fibrous remodeling as a consequence of chronic esophageal inflammation has been demonstrated in children and adults with EoE, and reproduced in animal models [204]. Eosinophil-associated tissue remodeling is a common process found in several conditions in which chronic eosinophilic inflammation is the common hallmark, including bronchial asthma [205], hypereosinophilic syndrome [206], eosinophilic gastroenteritis [207], and lastly, EoE [204]. All share structural changes within the affected tissue, including subepithelial fibrosis, which ultimately alter the functionality of the affected organs. Uncontrolled remodeling due to ongoing inflammation in EoE may adversely affect esophageal function, leading to dysmotility [208], esophageal rigidity [209], progressive dysphagia and food impaction and, finally, stricture formation.

Esophageal strictures constitute one of the most severe complications of EoE that develop as a result of a long-standing untreated eosinophilic inflammation. Despite patient age and delayed diagnosis being recognized as determining factors for fibrotic esophageal strictures [210–212], not every patient with prolonged EoE evolution develops such strictures. Esophageal strictures are less commonly found in pediatric cases of EoE, likely due to the limited progression of the disease.

7.1. Cellular & molecular basis of tissue remodeling in EoE

Several mediators released from inflammatory cells are involved in driving esophageal remodeling in EoE, with a particular role for transforming growth factor (TGF)-β1 [213], analogous to the one observed in airway remodeling associated with asthma [214]. In addition to TGF-β1 signaling, other mechanisms involved in EoE remodeling include epithelium-mesenchymal transition and angiogenesis [215].

Research conducted in a murine model [216] and on esophageal cell cultures [217] has shown that subepithelial fibrosis in EoE develops as a consequence of IL-5, IL-4 and IL-13-promoted tissue eosinophilia [218,219]; blocking its respective activation pathways represents potential therapeutic targets. The esophageal tissue of EoE patients shows higher levels of angiogenic factors compared with control samples including CD31, von Willebrand factor, VEGF-A and vascular cell adhesion molecule-1, all of which promote neovascularization and angiogenic remodeling [220]. An activated endothelium facilitates the arrival of bone marrow-derived inflammatory cells into the esophagus, which are activated to release their granule proteins locally. Eosinophils and other proinflammatory cells interface with mesenchymal cell components in the deep esophageal layers, affecting fibroblasts and muscle cells by making them direct targets of activated eosinophils and their products [217]. Fibrosis in EoE has been related with eosinophil activation [221] which can be determined by immunohistochemical staining for eosinophilic major basic protein (MBP) [158]. Eosinophil-released MBP increases the expression of *FGF-9* in biopsies of EoE patients [222], correlates with the basal cell hyperplasia in the esophageal epithelium, and directly promotes both fibroblast activation and deposition of extracellular matrix (ECM) (Figure 1). Eosinophils also produce and secrete high amounts of CCL18, a type 2 chemokine implicated in fibrous remodeling of the lungs, through fibroblast proliferation and collagen deposition. High expression levels of this chemokine have been shown in EoE [223].

7.2. Epithelial mesenchymal transition in EoE

Epithelial mesenchymal transition (EMT), a process characterized by activating quiescent epithelial cells and fibroblasts, causing them to transdifferentiate into myofibroblasts, and defined by gain of mesenchymal markers (such as α-*smooth muscle actin* and *vimentin*) and loss of epithelial (*E-cadherin*) gene expression, has been recognized as a key process in all models of fibrosis [224]. TGF-β released from activated eosinophils and mast cells [225] strongly induces EMT in the esophageal epithelium [215] and is the most extensively analyzed cytokine in EoE-associated fibrous remodeling. In addition, EMT in EoE can also occur independently of TFG-β but mediated by IL-1β and TNFα as previously implicated in other models of cross-talk and fibrosis [226].

Myofibroblasts share features of both fibroblasts and smooth muscle cells and simultaneously participate in the synthesis, deposition and degradation of ECM along with the contraction of wound tissue [227]. Tissue remodeling also involves morphological and functional changes in smooth muscle components. In fact, esophageal muscle cells respond to various profibrogenic stimuli and eosinophil products. Thus, while MBP is a strong agonist of the M2-type receptors of acetylcholine, which governs smooth muscle function [228], at the same time, eosinophil-derived mediators affect the release of acetylcholine from the neuromuscular junction [217]. Hypertrophy of the muscularis mucosa along with the circular and longitudinal muscle layers has also been reported in patients with EoE [229], contributing to the esophageal dysfunction repeatedly demonstrated in EoE patients of all ages.

7.3. Clinical assessment of esophageal remodeling in EoE

As a result of fibrous remodeling, alterations in the biomechanical properties of the esophageal wall are common features of EoE [230]. The distensibility of the esophageal body was significantly reduced compared to controls in patients with EoE when assessed using the EndoFLIP system (Crospon Medical Devices, Galway, Ireland) [231], which uses impedance planimetry to calculate multiple adjacent cross-sectional areas within a cylindrical bag while simultaneously measuring intraluminal pressure during controlled volumetric distension [232]. EndoFLIP research in EoE has shown that a reduced esophageal distensibility predicts the risk of food impaction [233] and correlates with endoscopically-identified ring severity [234]. Improvements in esophageal body distensibility are achieved with medical and dietary therapies without dilation [235]. However, a lack of correlation between eosinophil counts and esophageal distensibility has been shown with EndoFLIP [233], partially explaining the dissociation between inflammatory activity and symptoms in EoE. Whether the addition of the EndoFLIP system to patient reported outcome measures can enhance the accuracy of predicting the biological activity of EoE and improve results of EoE therapies, including endoscopic dilation, warrants further investigation [236].

7.4. Therapeutic interventions for EoE-associated fibrous remodeling

Mechanical dilation with through-the-scope hydropneumatic balloons and Maloney or Savary bougies constitutes a preferred treatment option for EoE patients with esophageal strictures or a narrow-caliber esophagus, which improves dysphagia in 95% of patients, according to a recent meta-analysis including 27 studies assessing 845 individual patients undergoing 1,820 dilation procedures [237–239]. Because endoscopic dilation is a mechanical procedure with no effect on the underlying inflammatory process [238], its efficacy is limited over time, with duration of the effect ranging from 1 to 36 months [237].

Swallowed topical steroids have been demonstrated effective to reverse fibrous remodeling in children and adults with EoE, as well as in reducing the consequences of fibrosis in the esophageal distensibility. Research in children documented first that collagen deposition was a reversible phenomenon [188,239,240]. Reduction in epithelial eosinophils was a predictor of resolution of remodeling that accounted, in parallel, for the reduction in TGF-b and pSmad 2/3-positive cells and decrease in vascular activation, as determined by reduced expression of vascular cell adhesion molecule-1 [188]. Subsequent research in adult patients showed that fluticasone propionate use for one year were also able to non-significantly reduce collagen deposits in the esophageal subepithelium despite the treatment induced down regulation of profibrogenic cytokine gene expression [223]. In contrast no changes were noted with low doses of budesonide [241]. The

fact that the drug formulas used were not designed for esophageal targeting or insufficient amounts for esophageal covering were applied might explain the difference among ages. The effectiveness of novel formulas of budesonide specifically developed for EoE [242] in reducing subepithelial fibrosis is yet to be determined.

As for dietary therapy, studies in adult patients have shown its effectiveness in reversing clinical, endoscopic, and histologic features in EoE [120,243,244], but suggest that fibrostenotic phenotype may be less likely to respond [243].

Both elimination diet and topical steroid therapy may improve esophageal distensibility using FLIP together with reducing esophageal eosinophilia [235]. The lasting effect on esophageal distensibility to a complete esophageal recovery is yet to be determined.

Among the investigational therapies in fibrous remodeling, losartan, an angiotensin II receptor blocker approved to treat high blood pressure in children and adults, which has proven safe when administered to patients with normal blood pressure, is currently been tested for EoE. Losartan may reduce the amount of TGF-β thus constituting a potential treatment for fibrosis in EoE. A Phase II trial with increasing doses of losartan is currently underway to evaluate endoscopic, histological and symptomatic improvement [245]. An additional open-label study will assess changes from baseline in peak esophageal eosinophil count and in blood and esophageal TGFβ levels at the end of treatment [246].

8. Expert commentary

Early diagnosis of patients with EoE, providing them with effective therapies and developing non-invasive monitoring methods are currently the most relevant goals for clinicians. Identifying the specific risk factors for developing EoE and defining their relative weight is key to proposing future preventive strategies in populations at risk.

The relative contribution of genes and the environment in the origin of EoE has been analyzed by some studies with different approaches, all assigning a predominant role to the latter [180,184]. The environmental risk factors leading to EoE and the way they interact with the host toward losing immunological tolerance in the esophageal mucosa are still to be revealed [192,203]; its discovery is essential to propose preventive strategies for EoE. The underexplored potential role of esophageal microbiota in mediating the interplay between the environment and the esophageal mucosal surveillance system appears as one the most promising approaches. Changes in the esophageal microbiome composition in adult and pediatric EoE patients compared to non-EoE controls have also been recently described [247,248] while antibiotic-induced changes in the microbiota represents an early life risk factor for developing EoE [192]. Biopsy samples from adults with active EoE have increased bacterial load by 16S expression and upregulation of several TLRs compared to controls which reverse after dietary therapy. Mediators of inflammation in the TLR signaling pathways were also upregulated. Finally, innate immune effector proteins also showed increased activity. All of these corrected after disease remission induced by a dietary intervention [50]. Genotyping of nucleotide polymorphisms (tSNPs) revealed TLR3 as a novel genetic susceptibility locus for developing EoE, with independent effects of TSLP [187].

The discrepancy between symptoms and histopathologic features is one of the major challenges in patients with EoE. Significant esophageal eosinophilia can be present in many patients with minimal symptoms due to food behavior adaptations, and some patients under histologic remission may suffer food impaction episodes due to a reduced esophageal caliber. Endoscopy with biopsies is essential for the initial diagnosis of EoE and the only accurate method for disease monitoring [1]. Identifying reliable non- or minimally invasive markers for EoE is, therefore, urgently required. Several candidate single molecules obtained mainly from blood have been studied in patients with EoE, none of them having provided enough accuracy to be incorporated into clinical practice [249]. However, efforts to identify new EoE biomarkers have rapidly expanded to include complex combination of molecules which could provide a reliable distinction of active EoE from inactive EoE, and both from normal controls and atopic subjects. In fact, a well-preserved EoE transcriptome has facilitated the development of an EoE diagnostic panel that provides the additional advantage of identifying histologically ambiguous subjects who may later develop active EoE [67]. The utility of such a panel to elucidate key elements in EoE, including the potential responsiveness to drug-based or dietary therapies, predicting the disease course, or in identifying atopic patients or relatives at risk of developing EoE, is a potential utility that should be assessed [250].

9. Five-year view

The expansion of EoE and its wide recognition across multiple settings will undoubtedly facilitate in coming years significant advances in the knowledge of the intimate mechanisms of the disease, in the optimization of diagnostic and disease monitoring methods to make them less dependent on endoscopy, and in its therapeutic approach toward personalized medicine.

Minimally invasive methods for patient diagnosis and monitoring are urgently needed in clinical practice; and some preliminary approaches have provided promising results. Among them, substituting endoscopy with biopsies by cytology have been assessed recently. The cytosponge consists of an ingestible gelatin capsule comprising compressed mesh attached to a string, able to obtain cells from the esophageal surface when removed. Its accuracy compared to endoscopy with biopsies has been recently assessed in a multicenter study, which provided a sensitivity and specificity of 75% and 86%, respectively (AUC 0.87) for disease activity, defined by a cutoff of 15 eos/HPF. No complications were reported, and patients preferred cytosponge to endoscopy as a monitoring method [251].

An alternative approach to cytology is to retrieve eosinophil-derived proteins obtained from esophageal exudates. A minimally invasive string-based technology composed of a capsule filled with 10 cm of string, derived from the Enterotest (HDC Corporation, Pilpitas, CA, USA) originally designed to detect gastric and small intestine pathogens, sample bile and assess for GERD was assessed in pediatric patients [252]. The quantities of eosinophil granule proteins in esophageal luminal samples obtained with the esophageal string test significantly correlated with eosinophil counts and granule protein levels in esophageal biopsies; MBP1 and Charcot-Leyden crystal protein indicated a high predictive

power with AUC of 0.97 and 0.97, respectively, compared to biopsies. More recently, the esophageal mucosa was sampled with a cytology brush inserted through a nasogastric tube. Eosinophil-derived neurotoxin (EDN) was measured by ELISA from the samples obtained with the brush, in the samples extracted from brushes and its diagnostic accuracy validated against endoscopic biopsies. A sensitivity of 0.98 and specificity of 0,89 was found, overall providing an AUC of 0.99 [253]. These novel methods suggest that eosinophil-derived proteins are superior to cytology in monitoring esophageal inflammation in patients with EoE.

Key issues

- EoE is a particular form of food allergy associated with a Th2-type inflammatory response that shares common molecular pathways with atopic diseases characterized by IL-5, IL-13 and eotaxins expression.
- The esophageal epithelium is being placed at the center of the pathogenesis of EoE: an impaired barrier function related to a depletion of SPINK7 determines an increased permeability, allowing an enhanced contact between mucosal immune system and component of the diet or microbiota.
- Epithelial cell-derived TSLP activates antigen presenting cells in EoE to polarize T cells toward a Th2-type response with secretion of IL-13.
- IL-13 upregulates CAPN1 in the esophageal epithelium, a protease with an important role for epithelial barrier function which is involved in repairing IL-13-induced epithelial changes. CAPN14 is also implicated in the down-regulation of DSG1.
- Several genes and variants providing susceptibility to EoE have been identified, overall contributing modestly to disease susceptibility. In contrast, environmental factors including perinatal and early life exposures are mainly involved in determining risk for EoE.
- Interactions of epithelial cells with components of the esophageal microbiota modulate the expression of CXCL16 and recruit invariant natural killer T (iNKT) cells toward the esophageal epithelium, in an early stage of EoE development.
- Mediators released from activated mast cells and eosinophils induce epithelial mesenchymal transition leading to esophageal remodeling by subepithelial deposition of collagen and other extracellular matrix components, the reversion of which is being increasingly recognized as a clinically relevant target for therapy.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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DISCUSIÓN

1.- Epidemiología de la EoE

Los resultados de nuestro estudio poblacional documentan un dramático y rápido aumento en la epidemiología de la EoE en nuestra región en los últimos 12 años. La prevalencia de la EoE en adultos calculada en un estudio anterior realizado por nuestro grupo realizado con datos de hasta 2012 fue de 44,6 casos por 100.000 habitantes, y la incidencia media anual fue de 6,4 nuevos casos por 100.000 habitantes y año[21]. En contraste, el trabajo actual muestras que la prevalencia de EoE al final del año 2017 fue de 111,9 casos por 100.000 habitantes (tanto en niños como en adultos), mientras que la incidencia media anual de 10,6 y 9,1 nuevos casos / 100.000 habitantes y año para niños y adultos, respectivamente.

Esto supone un aumento de la prevalencia de la enfermedad en más de 2,5 veces y casi dos veces en la incidencia media en un periodo tan corto como 5 años. Un trabajo reciente publicado por el Dr. Javier Molina-Infante había analizado la prevalencia de EoE en adultos de Cáceres para el año 2016, proporcionando cifras de 81,7 casos por 100.000 habitantes[27]. Sin embargo, nuestro trabajo proporciona las cifras de prevalencia más elevadas comunicadas en la literatura hasta la fecha para la EoE, superando cualquier estimación previa, y confirmando la tendencia global al aumento continuado en la frecuencia de la enfermedad[16,26,43,252].

La mayoría de los pacientes diagnosticados de EoE lo son entre los 20 y 24 años y entre los 35 y 39 años, siendo la prevalencia en estos grupos de edad más elevada que en el global (300 y 263 casos por 100.000 habitantes respectivamente), aunque cada vez más son diagnosticados también más casos en edad infantil (en torno a 160 casos por 100.000 habitantes entre los 5 y 14 años). Después de los 45 años se produce un dramático descenso en los casos diagnosticados de la enfermedad. Estos hallazgos confirman a la EoE como una enfermedad predominante de adultos jóvenes.

Al mismo tiempo, la incidencia y la prevalencia actual de la EoE para España central se igualan a la enfermedad inflamatoria intestinal para nuestro medio. Así, la incidencia comunicada para la enfermedad de Crohn en países europeos se encuentra entre 8,6 y 9,9 nuevos casos por 100.000 habitantes y año[253–255], mientras que la prevalencia para España ha sido calculada en 137,2 casos por 100.000 habitantes[253]. Tras estos datos, la EoE no debería considerarse más una enfermedad rara sino una causa prevalente de síntomas esofágicos, que está presente en uno de cada 893 españoles.

Las razones que subyacen a este aumento en la epidemiología de la EoE a nivel global no han sido completamente definidas, pero probablemente muchas coinciden con las que explican la expansión de muchas otras enfermedades de tipo inmuno-alergico. La primera de ellas tiene relación con el mejor conocimiento de la enfermedad y reconocimiento por parte de los clínicos. En efecto, los estudios epidemiológicos con que han reportado mayor frecuencia de EoE han sido realizados en regiones con investigadores y clínicos expertos en la enfermedad, incluyendo diversas áreas de Estados Unidos de América, España y Suiza. La segunda razón deriva de un reciente cambio conceptual en la definición de la EoE. Algunos estudios epidemiológicos previos habían excluido de sus cálculos a los pacientes respondedores a IBP, que pueden llegar a representar hasta la mitad de los casos. Múltiples pruebas científicas y la más reciente guía de práctica clínica basada en la evidencia no diferencian entre la EoE y la antes denominada PPI-REE, sino que ambas entidades forman parte de una misma entidad[1]. Es posible, por tanto, que algunos estudios anteriores hayan podido infraestimar la verdadera magnitud de la EoE. El aumento en el uso de la endoscopia hasta su generalización como técnica de primera línea en el estudio de múltiples trastornos digestivos ha sido otra de las razones esgrimidas por varios autores para explicar el aumento de la epidemiología de la EoE[256,257]. Sin embargo, estudios recientes, incluido el nuestro, demuestran que el crecimiento en el diagnóstico de nuevos casos de EoE supera al aumento continuo en el uso de la endoscopia[27,258]. Finalmente, la naturaleza crónica de la EoE y su nula mortalidad determinan un acúmulo de casos en una determinada región geográfica y el aumento continuo de su prevalencia. Sin embargo, el aumento continuado en la aparición de nuevos casos cada año (incidencia creciente) contribuye de uno modo relevante a la prevalencia creciente de la enfermedad.

Al aparecer preferentemente en paciente jóvenes que tienen una dilatada esperanza de vida en el momento del diagnóstico, la altísima frecuencia que alcanzará la EoE en las poblaciones de países desarrollados en las próximas décadas harán de ella un importantísimo problema de salud pública y un reto asistencial además de financiero. Su naturaleza crónica, la necesidad de implicar a un equipo multidisciplinar de profesionales (gastroenterólogos, dietistas, pediatras, patólogos, alergólogos, entre otros), la recurrencia de los síntomas tras cesar el tratamiento y la edad temprana de los pacientes en el momento de su diagnóstico (niños, adolescentes o adultos jóvenes) impactan directamente sobre los recursos de los sistemas de salud.

Un reciente estudio cuantificó el coste anual de la EoE en Estados Unidos en 1.400 millones de dólares[259], si bien no consideró las cifras actuales de prevalencia ni las tendencias temporales. Se hace, por tanto, esencial implementar estrategias preventivas de la enfermedad tras la identificación de sus factores de riesgo, así como optimizar las intervenciones más coste-efectivas.

Nuestro estudio no pudo documentar una tendencia estacional en el momento del diagnóstico de la EoE, siendo el número de pacientes diagnosticados durante las cuatro estaciones del año muy similar. Tampoco pudimos observado un efecto significativo de la estación de polinización en el número de nuevos casos identificados. Aunque algunos autores siguen defendiendo la influencia de las estaciones en la aparición de nuevos casos de la enfermedad[242,260–262], cada vez disponemos de más pruebas de calidad del nulo efecto que la estacionalidad posee sobre el riesgo de aparición de la enfermedad[27,263,264]. Entre ellas, una reciente revisión sistemática desarrollada por nuestro grupo concluyó en que no existía una estacionalidad para el momento del diagnóstico ni para la recurrencia de los síntomas en los pacientes con EoE[265]. Si podría existir, en cambio, un mayor reconocimiento de la enfermedad durante la primavera y el verano derivado de la oportunidad para alcanzar un diagnóstico de EoE en pacientes con síntomas de disfunción esofágica leves o moderados, que consultan por agudización estacional de sus manifestaciones atópicas asociadas, alcanzándose así el diagnóstico de una EoE que venían padeciendo de manera larvada.

Por otro lado, nuestro estudio ha analizado el tiempo de presentación de los síntomas de EoE desde antes del diagnóstico y, por tanto, del retraso diagnóstico en nuestro medio. Para nuestro estudio, el tiempo medio de evolución de la enfermedad fue de 36 meses (es decir, 3 años de media desde que el paciente comenzó a percibir sus síntomas hasta el momento del diagnóstico de EoE, pudiendo llegar a ser incluso de 30 años en algún caso). En contraste, el retraso diagnóstico (desde la primera consulta médica debido a los síntomas esofágicos hasta el diagnóstico de le enfermedad) se situó en aproximadamente 6 meses de media, siendo ambos tiempos menores a los comunicados por otros estudios[27,45,266,267]. Este hecho podría deberse a la limitada extensión de nuestra área de referencia, lo que facilita el acceso de los pacientes a las consultas médicas y al elevado conocimiento de la enfermedad por parte de los profesionales implicados en el diagnóstico de la misma en un centro de referencia.

Por último, como en la gran mayoría de los estudios realizados en EoE, se observó una mayor frecuencia de le enfermedad entre los varones respecto a las mujeres[18,21,22,40], con un ratio hombres : mujeres de 6,8 : 1. De hecho, si nos centramos solamente en los varones, podemos estimar unas cifras de incidencia media de 16,1 nuevos casos por 100.000 habitantes y año tanto en niños como en adultos y una prevalencia de 172 y 200 casos por 100.000 habitantes para niños y adultos respectivamente.

En resumen, la incidencia y prevalencia de la EoE en nuestra área ha aumentado rápidamente a lo largo de los últimos 12 años para los pacientes de todas las edades (tanto en niños como en adultos). La EoE afecta más frecuentemente a varones jóvenes (entre 20 y 39 años), observándose un aumento paulatino también en niños (5 – 14 años). No hemos encontrado pruebas de estacionalidad en el momento del diagnóstico de la enfermedad ni de que su aumento se deba al creciente número de procedimientos endoscópicos realizados a lo largo del periodo de estudio.

2.- Caracterización del infiltrado inflamatorio mastocitario

Hasta la fecha, nuestro estudio de caracterización del infiltrado inflamatorio mastocitario en la EoE es el más amplio análisis de esta población celular y su significación en la EoE.

2.1.- Densidad de células inflamatorias (eosinófilos y mastocitos), activación y fenotipo de los mastocitos

La EoE se caracteriza histológicamente por una densa infiltración inflamatoria del esófago donde predominan los eosinófilos y los mastocitos. Nuestros resultados confirman que la EoE se caracteriza por una mayor densidad mastocitos en comparación con los sujetos controles con esófagos normales. En nuestro estudio, el grupo de pacientes con EoE tuvo una media de eosinófilos intraepiteliales de 56,8 eosinófilos por cga y de 18,6 mastocitos intraepiteliales por cga en las biopsias esofágicas, mientras que ni eosinófilos ni mastocitos fueron identificados en el grupo control. El número de eosinófilos se correlacionó significativamente con el número de mastocitos en las muestras de biopsias esofágicas (rho = 0,808; $p<0,001$), y ambas con la puntuación de síntomas en los pacientes adultos con EoE (rho = 0,895 y 0,782; $p<0,001$, respectivamente).

La activación de los mastocitos se determinó mediante el nivel de expresión de sus proteasas específicas, principalmente triptasa, quimasa y carboxipeptidasa A3. El RNA mensajero de todas estas proteasas se encuentra sobre-expresado en los pacientes con EoE respecto a los controles (3,2 veces para quimasa y carboxipeptidasa y 1,7 veces para la triptasa), y además se documentó mediante inmunofluorescencia el aumento en la expresión de las proteínas. La densidad y la activación de los mastocitos se asociaron directa y significativamente, y el nivel de expresión de estas proteasas de mastocitos también se correlacionó significativamente con los síntomas de los pacientes.

Los mastocitos MC_{TC} fueron el fenotipo predominante en la EoE, representando más del 90% de los mastocitos del epitelio esofágico, y estando además presentes tanto en papila conjuntiva como en la lámina propia. Hasta la fecha ningún estudio había examinado el fenotipo de los mastocitos implicados en la enfermedad. De los dos tipos de mastocitos presentes en los humanos, los MT_T se encuentran predominantemente en la mucosa bronquial y alveolos, mientras que los MC_{TC} se encuentran principalmente en la piel, mucosa nasal, y submucosa intestinal. Estos segundos mastocitos no responden específicamente a los medicamentos estabilizadores de mastocitos como el cromoglicato sódico[182], lo que explica la ineficacia de estos fármacos en el tratamiento de la EoE.

El mecanismo más estudiado de la activación y degranulación de los mastocitos es el producido por la interacción de un antígeno con su anticuerpo IgE específico unido en la membrana celular, lo que activa el receptor de alta afinidad para IgE (FcεRI), conduciendo a una rápida liberación de mediadores, citoquinas y factores de crecimiento[268] que puede incluso desencadenar anafilaxia. Sin embargo, los pacientes con EoE no suelen presentar reacciones anafilácticas frente a los alimentos que causan la enfermedad, lo que esto indica que en la EoE deben existir otros mecanismos implicados en la activación de los mastocitos. De hecho, los mastocitos también pueden ser activados de forma independiente de IgE mediante sustancias del microambiente, como citoquinas, neuropéptidos y productos bacterianos, entre otros. De hecho los mastocitos MT_{CT} son buenos respondedores a estímulos reguladores no mediados por IgE, como por ejemplo los TLRs o incluso mecanismos no inmunológicos[233,234]. La proteína mayor básica (MBP) derivada de eosinófilos posee la capacidad de

activar mastocitos y promover su degranulación, un hecho que podría explicar la antes referida asociación entre la densidad de eosinófilos y mastocitos que se encuentra en esta enfermedad.

Hemos observado una asociación entre la densidad y el estado de activación de los mastocitos con la sintomatología de los pacientes. Muchos de los síntomas reportados por los pacientes indican alteraciones en la función motora de los pacientes y sugieren una disfunción del músculo liso esofágico. La literatura científica ha documentado repetidamente la capacidad de los mastocitos para inducir dismotilidad e hiperalgesia visceral en varias enfermedades inflamatorias, incluidas la propia EoE[229], por lo que parece que los mastocitos desempeñan un papel importante en el origen y la percepción de estos síntomas.

2.2.- Reclutamiento de mastocitos y eosinófilos

Mientras que el reclutamiento de los eosinófilos activados hacia los tejidos se realiza fundamentalmente mediante la acción de eotaxinas, los mastocitos son reclutados fundamentalmente por la acción de *stem cell factor* (SCF). Todas las eotaxinas humanas (eotaxina 1, 2 y 3) se encontraron sobre-expresadas en los pacientes con EoE respecto a los sujetos del grupo control, siendo eotaxina-3 la que mayor nivel de sobreexpresión mostró (más de 51 veces) seguida por la eotaxina-2 (más de 12 veces) y por la eotaxina-1 (más de 8 veces). Además, CCR3 (el principal receptor para las eotaxinas) también se encontró sobre-expresado en las muestras de pacientes con EoE en casi 4 veces respecto a los controles. Para los mastocitos, SCF y su receptor SCFR también encontraron sobre-expresados entre 5 y 4 veces respectivamente, sobre los niveles de las muestras controles.

La infiltración celular en el epitelio esofágico de los pacientes con EoE se ha correlacionado con la expresión de los principales factores quimioatrayentes, y se han descrito correlaciones significativas entre la expresión de la eotaxina-3 y la densidad de eosinófilos. Nosotros además documentamos una asociación significativa entre el nivel de expresión de SCF y la densidad de mastocitos en el epitelio esofágico. La expresión de las eotaxinas-2 y 3 se correlacionó significativamente con los síntomas clínicos de la EoE, al igual que la del propio SCF.

2.3.- Efecto del tratamiento dietético sobre la densidad, actividad de los eosinófilos y mastocitos y de sus moléculas reclutadoras

El tratamiento dietético con dietas de eliminación empírica de seis alimentos ha demostrado ser efectivo para inducir la remisión de la EoE. Nuestros resultados mostraron una reducción en la densidad de eosinófilos y mastocitos tras dicho tratamiento: la densidad de eosinófilos se redujo desde 56,8 a 3 eosinófilos por CGA y la de mastocitos de 18,6 a 1,4 células por CGA. El tratamiento dietético también redujo significativamente la sintomatología de los pacientes, la expresión de todas las eotaxinas y su receptor hasta los niveles de expresión del grupo control. También redujo de manera significativa la expresión del SCF y de su receptor, aunque este último no de manera significativa.

Además, la dieta de eliminación de seis alimentos revirtió la sobre-expresión de todas las proteasas específicas de los mastocitos (triptasa, quimasa y carboxipeptidas A3) tanto a nivel de expresión génica como a nivel de expresión proteica hasta el nivel del grupo control.

Nuestros datos indican que los mastocitos son un elemento fundamental en el infiltrado inflamatorio de la EoE que mantienen una estrecha interacción con los eosinófilos: Los segundos representan una importante fuente de activación de mastocitos y éstos son un elemento relevante en la inducción de los síntomas de disfunción esofágica. La densidad mastocitaria, la expresión de sus receptores y los niveles de síntesis de sus principales proteasas se relacionaron de manera directa con la intensidad de los síntomas, y éstos mejoraron tras la reducción del infiltrado mastocitarios mediada por tratamiento dietético. Es por tanto razonable proponer que los mastocitos representan una diana terapéutica potencial para aliviar los síntomas de la EoE que ha sido estudiada de forma muy limitada. De hecho, nuestros trabajos han proporcionado una explicación a la falta de eficacia del tratamiento con estabilizadores de mastocitos (cromoglicato y nedocromil) en la EoE, pues los mastocitos mayoritarios del esófago (MC_{TC}) no responden a esta terapia.

3.- Implicación de los TLRs en la fisiopatología de la EoE

El sistema inmune innato desempeña un papel crecientemente reconocido en la regulación de las interacciones entre la microbiota y la inmunidad del huésped. La alteración en estas interacciones ha sido involucrada en múltiples enfermedades de base inmunológica y alérgica[269]. Los TLRs representan uno de los mecanismos de reconocimiento de microbiota mejor estudiados, y su papel en múltiples enfermedades es motivo de investigación creciente. Si bien estudios preliminares han mostrado que la EoE podría asociarse a cambios en la composición cuantitativa y cualitativa de la microbiota esofágica, hasta ahora ningún estudio había analizado de manera específica la expresión de los principales TLRs en la EoE, la actividad de esta vía de activación del sistema inmune, ni el efecto del tratamiento efectivo sobre la misma.

3.1.- Expresión de los TLRs y carga bacteriana en la mucosa esofágica de los pacientes con EoE

El esófago es un órgano especialmente expuesto a múltiples antígenos de origen microbiano, alimentario e incluso aéreo, por lo que requiere de mecanismos específicos para proteger su mucosa, identificando y respondiendo específicamente frente a estímulos agresores, o tolerando la exposición a estímulos inocuos. Los diferentes receptores TLRs permiten al sistema inmune innato reconocer patrones moleculares conservados asociados a diferentes tipos de patógeno, y cada TLR responde a componentes bacterianos o víricos únicos, que en conjunto permiten la discriminación precisa del entorno microbiano luminal. En los últimos años, varios estudios habían investigado las vías de señalización mediadas por los TLRs en algunas enfermedades alérgicas[270,271]. Estas vías de señalización dependientes de TLRs regulan la respuesta inmune y están conectadas a la actividad del receptor de alta afinidad para IgE (FcεRI) expresado en mastocitos, actuando como conector entre el sistema inmune innato y el sistema inmune adaptativo.

En el asma bronquial TLR2, TLR4 y TLR9 desempeñan un papel predominante[272,273], El asma bronquial y la EoE muestran numerosas similitudes, incluyendo una respuesta inmune tipo Th2 alterada, la implicación de eosinófilos y mastocitos en su patofisiosología, la inflamación transmural que promueve disfunción del músculo liso y remodelación

fibrosa, una respuesta clinicopatológica a esteroides tópicos y su control al evitar la exposición a los antígenos responsables. Pero hasta la fecha ningún estudio había evaluado la expresión y el papel potencial que los TLRs podrían desempeñar en la EoE, ampliando así los estudios que han comenzado a definir la funcionalidad de estos TLRs en el tracto gastrointestinal.

Nuestros resultados muestran una expresión génica de TLR1, TLR2, TLR4 y TLR9 entre 3 y 4 veces más en las biopsias de mucosa esofágica de los pacientes adultos con EoE respecto a los controles. Esta sobre-expresión génica también se ve confirmada por la expresión a nivel proteica medida mediante inmunofluorescencia. Sin embargo, no observarnos cambios en la expresión de TLR3 y TLR6.

TLR1 responde a los lipopéptidos triaciles y TLR2 al ácido lipoteicoico y peptidoglicanos, siendo todos componentes de la pared bacteriana. Ambos están implicados en reducir la activación del FCεRI, lo que reduce la degranulación de los mastocitos mediada por IgE[273,274]. TLR4 es estimulado por los lipopolisacáridos presentes en las bacterias gran negativas, aunque puede ser activado por algunos otros alérgenos que muestran homología estructural mediante un mecanismo de mimetismo molecular[275]. Pero en contraste con TLR1 y 2, la activación de TLR4 aumenta la actividad de FCεRI y promueve la expresión de citoquinas tipo Th2 implicadas en las respuestas eosinofílicas[276]. En condiciones basales, la expresión de TLR4 se encuentra reducida en la mucosa bronquial en relación a la expresión de TLR2, siendo el ratio TLR 2/4 el que define la activación final del FCεRI[277,278]. En el caso de la EoE, nuestros pacientes mostraron una expresión de TLR2 diez veces superior a la de TLR4, reforzando la hipótesis de que la IgE no desempeña un papel relevante en la enfermedad. De hecho las últimas investigaciones atribuyen un papel muy limitado a la IgE en la fisiopatología de la EoE en favor de la IgG4[279–282], y aunque se ha observado que IgE se une a los mastocitos en el epitelio esofágico de pacientes atópicos con EoE[283], este hecho no constituye una ruta de activación, ya que los pacientes con EoE no desarrollan respuestas inflamatorias rápidas después de exponerse a los alimentos que les producen la enfermedad ni el tratamiento con anticuerpos anti IgE resulta efectivo para la resolución clinicopatológica de la enfermedad[284].

Por último, TLR9 es un receptor intracelular activado por ADN bacteriano rico en islas CpG, que una vez activado promueve una respuesta inmune tipo Th1 con un aumento en la expresión de IFN-α y β. La estimulación del FCεRI por alérgenos suprime la activación del TLR9 con la consiguiente reducción de la respuesta Th1 y aumento de la respuesta inmune tipo Th2 típica de la EoE.

Además, nuestro estudio mostró que la carga bacteriana en las muestras de esófagos de los pacientes adultos con EoE era mayor que la hallada en los controles, confirman resultados previos reportados en una cohorte pediátrica[250]. La determinación de la carga bacteriana se realizó mediante la cuantificación relativa de el RNA ribosómico bacteriano S16. Si bien este método es simple y no permite valorar diferencias cualitativas en la microbiota, si aporta una prueba adicional al papel de ésta en el desarrollo de la EoE.

3.2.- Expresión de Mucinas

En condiciones normales, los componentes de la microbiota no suelen contactar directamente con el epitelio, sino que suelen estar embebidos en una capa mucosa compuesta por diversas mucinas[285]. El aumento en la expresión de RNA ribosómico 16 S en las biopsias epiteliales podría, por tanto, reflejar la pérdida de mucinas de la superficie esofágica como consecuencia de la inflamación de la superficie epitelial, en lugar de un verdadero aumento de la carga bacteriana. Nuestro siguiente paso, por tanto, fue determinar el nivel de expresión de mucinas en las muestras de estudio. Nuestros resultados mostraron que las mucinas Muc1 y Muc5B se encontraban inhibidas en su expresión (en concreto Muc5B se encontró inhibida 21,5 veces respecto a los controles), verosímilmente como reflejo de la disfunción de las células epiteliales que afecta a la integridad de la mucosa y aumenta su permeabilidad, exponiéndose de este modo a los componentes bacterianos y produciendo la activación del sistema inmune innato medida da por TLRs. En cambio, la expresión de mucina Muc4 se encontró sobre-expresada más de 7 veces en los pacientes con EoE, probablemente como un mecanismo compensatorio de la inhibición de las otras mucinas, lo que podría sugerir que la integridad de la mucosa se encuentra parcialmente preservada, tratando de limitar el contacto directo de la microbiota con la superficie de la mucosa esofágica.

Nuevos estudios son necesarios para corroborar este aspecto, en los que nuestro grupo está actualmente trabajando.

3.3.- Vías de señalización y mediadores

La carga de microbiota, la expresión de TLRs y de varias mucinas se mostraron alteradas en las muestras de pacientes adultos con EoE. Para conocer la funcionalidad de estos TLRs a continuación estudiamos sus vías de señalización y sus mediadores. Existen dos vías distintas de señalización asociadas con TLRs, una primera que requiere la proteína adaptadora MyD88, y otra MyD88-independiente. Finalmente ambas vías activan al factor transcriptional NF-κB que después de entrar en el núcleo celular, induce la producción de citocinas inflamatorias como IL-1, IL-8, TNF-alfa, e IL-12[286].

En nuestro estudio, todos los TLRs sobre-expresados en las biopsias de pacientes con EoE utilizan de un modo común la vía dependiente de MyD88, aspecto que queda confirmado por el hecho de que tanto MyD88 como NF- κB duplicaron su nivel de expresión en los pacientes adultos con EoE activa, confirmando así la funcionalidad de los TLRs y de sus vías de señalización.

Para confirmar estos mecanismos de señalización, también se estudió la expresión de las principales citoquinas inducidas por la acción de NF-κB, y pudimos demostrar sobreexpresión de IL-1β, IL-6, IL-8 e IL-10. De hecho, IL-8 fue la citoquina más sobreexpresado (más de 12 veces respecto a los controles), seguida de IL-10 (casi 7 veces más de expresión). Sin embargo, no observamos cambios en la expresión de IL-1α ni de TNF-α.

3.4.- Efectores de la respuesta inflamatoria

Como consecuencia de esta activación inmunitaria observada en la mucosa de los pacientes con EoE, posteriormente estudiamos los cambios en la expresión de los principales efectores del sistema inmune innato como PRF-1, iNOS, GZMA y GZMB. Concluimos que todos ellos excepto GZMB estaban sobreexpresados en los pacientes con EoE, siendo la molécula iNOS la que alcanzó la mayor diferencia en comparación con los controles.

3.5.- Sistema NLG2D

Por último, analizamos la expresión del sistema NK-G2D, midiendo para ello la expresión de los genes IL-15, MICA, MICB y KLRK1. Observamos que todos ellos, excepto MICA, presentaron una expresión significativamente más alta en las muestras de los pacientes con EoE activa en comparación con los sujetos controles, todos ellos entre 2 y 3 veces más elevados.

Las moléculas MICA y MICB son expresadas en la superficie celular, se corresponden al acrónimo del inglés “MHC class I chain-related genes”. Mientras que la expresión de moléculas de clase I del MHC es indicativa de integridad celular, las moléculas MICA y MICB se expresan en situaciones de estrés celular y evocan a una respuesta inmunitaria. Los genes MICA, y posiblemente también los otros genes MIC, han sido seleccionados para funciones especializadas que pueden ser innatas o adquiridas.

Las células citotóxicas expresan un conjunto de receptores activadores que incluyen, entre otros, a los Natural Killer Group 2D (NKG2D). NKG2D es un receptor activador que se encuentra expresado en la membrana de las células NK y linfocitos T CD4+ y CD8+. En las células NK activadas, NKG2D actúa como un primer receptor activador, por lo que él mismo es suficiente para activar la citotoxicidad mediada por células NK. En contraposición, NKG2D parece actuar como receptor co-estimulador en los linfocitos T CD8+, requiriendo otras señales para la activación completa de estas células efectoras.

3.6.- Efectos del tratamiento dietético

El tratamiento con dieta de eliminación de seis alimentos redujo a niveles similares a los controles la expresión de todas las moléculas sobreexpreasadas en las biopsias del esófago de los pacientes con EoE.

3.7.- Activación del sistema inmune innato en la mucosa del duodeno

Una vez comprobada la sobre-expresión de los TLRs y su funcionalidad y por tanto la activación del sistema inmune innato en el esófago de pacientes con EoE, se estudió si esta activación era específica del esófago o si también se produce en otros tejidos gastrointestinales sin inflamación, como es el caso de la superficie mucosa del duodeno.

Sorprendentemente, prácticamente los mismos TLRs que se encontraban sobreexpresados en las muestras del esófago, también se encontraron sobreexpresados en las de duodeno, a excepción del TLR9, aún no existiendo inflamación histológica en la mucosa del duodeno. Si bien los niveles de sobreexpresión en el duodeno eran inferiores a los del esófago aunque su diferencia respecto a controles estadísticamente significativa, el tratamiento con dieta de eliminación de 6 alimentos también redujo la expresión a los niveles normales.

Sin embargo, el estudio de las muestras duodenales mostró que tanto la carga bacteriana como la expresión de mucinas tenían niveles de expresión normal. Además, los TLRs sobreexpresados en duodeno parecen no ser funcionales, puesto que sus vías de señalización estaban inactivas, con sus factores transcripcionales, citoquinas mediadoras, moléculas efectoras de la inmunidad innata y sistema NK-G2D todos sin alteraciones en su nivel de expresión como si ocurría en el esófago.

3.8.- Diferenciación entre esófago y duodeno

Nuestros resultados han revelado un perfil de expresión génica específico de esófago que parece ser diferente al del duodeno. De hecho, mediante el análisis multivariante y el análisis de componentes principales (PCA) se pudieron diferenciar perfiles de expresión diferentes entre los esófagos de pacientes con EoE activa, EoE en remisión y sujetos controles. Sin embargo, esta diferenciación no pudo reproducida utilizando los perfiles de expresión génica específicos del duodeno. Aunque los TLRs se encuentren sobre-expresados a través del tracto gastrointestinal superior en los pacientes con EoE, sus vías de señalización únicamente serían funcionales en el esófago, manteniendo la respuesta inmune restringida a este órgano.

Se podría especular que una sobreexpresión de TLR en segmentos no inflamados del tracto gastrointestinal de pacientes con EoE podría ser paralela al aumento de citoquinas proinflamatorias también en tejido no inflamado como ocurre en pacientes con enfermedad inflamatoria intestinal[287].

La cuestión sigue siendo, sin embargo, por qué si los TLRs también se sobreexpresan en la mucosa no inflamada de pacientes con EoE, la enfermedad se restringe al esófago. Una posibilidad es que el aumento de la carga microbiana asociada a la mucosa (o su actividad metabólica) en el esófago (pero no en el duodeno) pueda ser el desencadenante de la inflamación, ya sea como un efecto directo o imitando los componentes de la dieta.

Nuestro estudio examina por primera vez el papel potencial de los TLRs en la patofisiología de la EoE. La EoE activa se caracteriza por una sobre-expresión de los TLRs, los factores de transcripción implicados en sus vías de señalización, citoquinas y efectores. Todos estos resultados ponen de manifiesto la activación del sistema inmune innato en los pacientes con EoE activa, sugiriendo una importante implicación y participación en la patogénesis de la enfermedad.

En resumen, el esófago de los pacientes adultos con EoE contiene una mayor carga bacteriana que, junto con una alteración en la capa mucosa, aumenta la expresión de los TLRs, que a su vez activan su cadena de transducción de señales, activando a su vez el sistema inmune innato y produciendo un aumento en la expresión de sus citoquinas específicas. Estos fenómenos se localizaron exclusivamente en el esófago, aunque prácticamente los mismos TLRs están sobre-expresados en el duodeno de los mismos pacientes, aunque en este segundo tejido éstos parecen no ser funcionales ya que la expresión de los componentes que se han medido no se ha visto alterada y, por tanto, no se produce inflamación en la mucosa del duodeno. Sin embargo, los mecanismos exactos que median las complejas interacciones entre la microbiota esofágica, el sistema inmunitario innato y las respuestas inflamatorias específicas de los alimentos en la fisiopatología de la EoE exigen futuras investigaciones adicionales.

Nuestro grupo ha abordado profundizar en el estudio de la función de la activación del sistema inmune mediada por TLR en pacientes con EoE, mediante proyectos que siguen 3 líneas complementarias:

a) Por un lado, estamos estimulando muestras de biopsia de pacientes con EoE en remisión con diferentes ligandos de TLR, tratando de reproducir las respuestas moleculares características de la activación de sus vías de señalización.

b) Por otro lado, estamos caracterizando las distintas células que expresan cada uno de los TLR en el esófago de los pacientes con EoE mediante el uso de tinciones inmunofluorescentes con co-localización, así como las células responsables de la expresión de mediadores y efectores de la respuesta inflamatoria. De este modo, tratamos de definir la localización de estos mecanismos dentro del epitelio esofágico.

c) Finalmente, mediante secuenciación del RNA ribosómico 16S, tratamos de definir los cambios específicos en la microbiota esofágica a nivel cualitativo que se producen en la EoE activa, y su posible reversión mediante diferentes tratamientos. De esta manera podremos identificar factores microbianos específicos que median la disfunción inmunológica que caracteriza la EoE.

4.- Nueva hipótesis integrativa de la fisiopatología de la EoE y sus mecanismos celulares y moleculares

La EoE es una enfermedad fisiopatologicamente compleja en la que interactúan múltiples mecanismos que involucran un gran número de células, moléculas y genes. La EoE surge de una respuesta inmunológica de tipo Th2 frente a componentes de la dieta, en la que IL-5, IL-13 y eotaxinas desempeñan funciones centrales en el reclutamiento y activación de los eosinófilos, en la disfunción del epitelio del órgano, y en la promoción de fenómenos de cronificación de la respuesta inflamatoria.

El reclutamiento de eosinófilos hacia el esófago responde al efecto de varias señales de activación liberadas por el tejido inflamado, que primero inducen la adquisición de propiedades funcionales específicas del tejido en los propios eosinófilos durante su circulación en sangre, que son diferentes dependiendo del tejido donde vayan realizar sus funciones inflamatorias (sean éstos la mucosa esofágica, bronquial o colónica) y el estado de actividad de la enfermedad[288].

A pesar del efecto de las moléculas de *homing* en el reclutamiento de eosinófilos hacia la mucosa esofágica, estas aún no se han evaluado en profundidad en la EoE. Sin embargo, conocemos que los eosinófilos circulantes en la sangre en EoE exhiben una expresión aumentada de CCR3, principal receptor de las eotaxinas[289], del receptor de baja afinidad para IgE (CD23), la molécula de adhesión intercelular (ICAM)-1 (o CD54), de la integrina CD11c, del receptor para la prostaglandina D2 CRTH2 y del RNAm de FOXP3[290].

Recientemente, las células epiteliales se han postulado como células relevantes en el inicio de la enfermedad, al haber sido reconocidas como uno de los principales componentes del sistema inmune innato desempeñando un papel central en la función defensiva de la mucosa del tracto gastrointestinal[291]. Se ha descrito que las células epiteliales de la mucosa esofágica son capaces de expresar moléculas del complejo mayor de histocompatibilidad (MHC) de clase II durante la inflamación y comportarse como células presentadoras de antígenos[292,293].

En condiciones fisiológicas el epitelio esofágico es una superficie relativamente impermeable que no permite el paso de moléculas de tamaño mediano y grande, produciendo así el efecto de barrera epitelial esofágica. En la EoE la infiltración eosinofílica es más abundante en los estratos más superficiales del epitelio, aquellos en contacto con la luz esofágica, y por tanto el punto de contacto con los alérgenos ingeridos, pudiéndose observar microabcesos de eosinófilos en estas capas[294].

Sin embargo, la EoE activa se caracteriza por una deficiente función de barrera epitelial, con expresión reducida de E-cadherina, desmogleina-1, involucrina y filagrina, todas ellas proteínas estructurales involucradas en el mantenimiento de la integridad de la mucosa. Otro hallazgo que apoya la pérdida de la función barrera del epitelio en la enfermedad es la expresión alterada de algunos componentes de las uniones estrechas como la claudina-1, claudina-4, claudina-7 y ocludinas[295–297]. Estas uniones estrechas son complejos de unión multiproteícos que evitan la salida de agua y solutos.

Como consecuencia de esta disfunción de barrera del epitelio se produce un aumento de la permeabilidad en la mucosa esofágica de los pacientes con EoE, permitiendo que los patógenos y antígenos de la dieta se introduzcan entre las células del epitelio esofágico, perpetuando y cronificando la inflamación del esófago característica de la EoE mediante diversas vías incluyendo las vías de señalización dependientes de TLRs, como se ha demostrado en el anterior apartado.

El epitelio esofágico también es la principal fuente de TSLP, citoquina clave en la EoE. La TSLP es producida principalmente por células no hematopoyéticas como las células epiteliales, fibroblastos y diferentes tipos de células estromales, y su mayor expresión está vinculada a muchas enfermedades alérgicas e inmunitarias, como el asma, la dermatitis atópica y la enfermedad inflamatoria intestinal[298–300]. Los factores que inducen la activación de la liberación de TSLP no están claramente definidos, pero juegan un papel importante en la activación de las células presentadoras de antígenos, incluidas las células dendríticas que captan, procesan y presentan antígenos alimentarios en la mucosa esofágica a las células T, promoviendo su maduración y secreción de citoquinas tipo Th2, incluida la IL-13 que se encuentra sobreexpresada en la EoE. De hecho IL-13 induce la expresión de eotaxinas tanto modelos murinos como cultivos celulares humanos, lo que conduciría al reclutamiento de eosinófilos, además de reproducir el transcriptoma característico de la EoE[301,302]. IL-13 también promueve la disfunción epitelial en la EoE disminuyendo la expresión de filagrina, involucrina, desmogleina 1 y proteínas de uniones estrechas de las células epiteliales[223,303].

Cuando durante las primeras etapas de la vida no se produce un contacto con componentes microbianos en su cantidad o proporción adecuada (ya sea por falta de exposición o por el uso de antibióticos durante los primeros años) se induce una acumulación excesiva y persistente de de células iNKT[304], haciendo que estos tejidos sean más susceptibles a posteriores estímulos. Las células iNKT están involucradas en una gran variedad de respuestas inmunes en diversas enfermedades alérgicas, y son capaces de producir y secretar rápidamente citoquinas de tipo Th2 como IL-4, IL-5, IL-13 y eotaxinas. Las células iNKT reconocen glicolípidos localizados en bacterias patogénicas presentados por la molécula CD1d pero también pueden ser activadas por esfingolípidos encontradas en los alimentos como la leche y los huevos. De hecho, los esfingolípidos de la leche han mostrado capacidad para activar iNKTs sanguíneas periféricas en pacientes con EoE[236]. En animales se ha demostrado que la activación de iNKT es suficiente para inducir EoE experimental[237], mientras que ratones deficientes en CD1d están protegidos para el desarrollo de la EoE experimental[305]. Los pacientes con EoE muestran un aumento de la densidad de células iNKT con respecto a los controles. CXCL16 es la principal citoquina responsable de la quimiotaxis de iNKT, por lo que la modulación del eje CXCL16-INKT-CD1d puede convertirse en una diana terapéutica potencial para ser investigada en la prevención y el tratamiento de la EoE.

Recientemente, se ha propuesto un papel cada vez más importante de la IgG4 en la EoE, al haberse demostrado una mayor concentración de IgG4 en pacientes en comparación con los controles y un aumento de la IgG4 especifica frente a alimentos como leche, trigo, huevo y frutos secos, que son los que más frecuentemente producen la enfermedad[306]. Los niveles de IgG4 total se correlacionan con el recuento de eosinófilos y expresión de IL-4, IL-10 y IL-13 en pacientes con EoE[279].

Los mastocitos como parte de la inmunidad innata, actúan contra parásitos y bacterias. Varios estudios previos habían demostrado su activación y mayor densidad en los pacientes con EoE[69,229]. La densidad de los mastocitos y su actividad se correlaciona con la infiltración eosinofílica dentro del epitelio esofágico[307] y con la severidad de los síntomas, mientras que los tratamientos con esteroides tópicos[308] y con dietas reducen la densidad mastocitaria. Además, también se ha publicado la expresión aumentada de varios mediadores específicos de los mastocitos como TGF-β1 que contribuye a la dismotilidad esofágica a través de la hipertrofia e hiperplasia del músculo liso, lo que contribuye a la dismotilidad y a los síntomas esofágicos.

Sin embargo, la activación de mastocitos en la EoE no parece estar mediada por la IgE, ya que cuando se produce por esta vía suele conducir a reacciones anafilácticas. Estas reacciones sistémicas inmediatas frente a los alimentos responsables no se describen en los pacientes con EoE, lo que indica que otros mecanismos independientes de IgE son los que producen la activación de los mastocitos, punto que nuestros estudios han demostrado, poniendo de relieve las vías de activación mediadas por TLRs.

En cuanto a la remodelación fibrosa del esófago de los pacientes con EoE debido al depósito subepitelial de colágeno como consecuencia de la inflamación crónica del órgano, ésta es la causa de la reducción del calibre y de las estenosis del esófago en esta enfermedad. En esta remodelación del esófago participan diversas moléculas como TGF-β1 (molécula involucrada también en la remodelación fibrosa en el asma), aunque otros mecanismos y moléculas también son relevantes para esta remodelación fibrosa, como se demuestra en modelos de ratones y cultivos celulares.

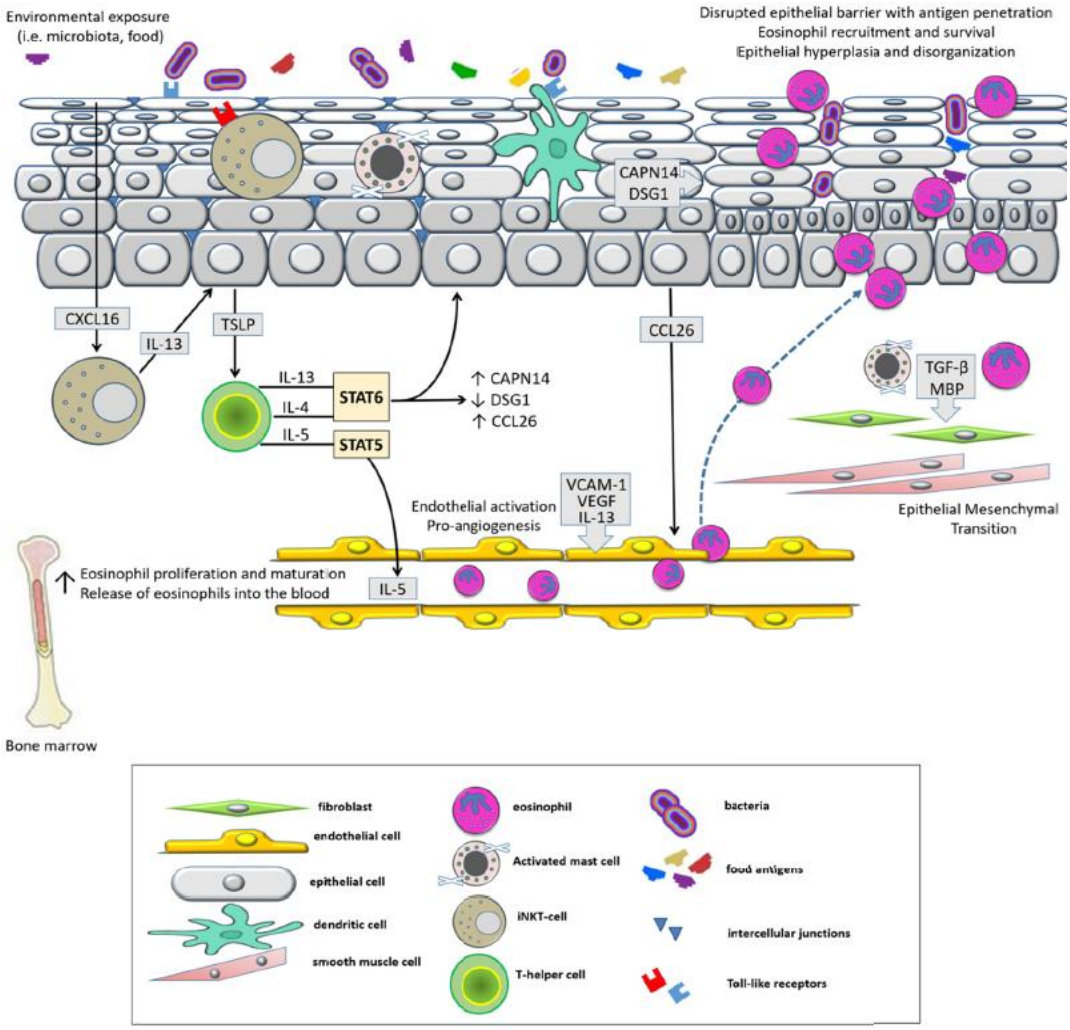

Figura 8: Nueva hipótesis integrativa de la fisiopatología de la EoE y sus mecanismos celulares y moleculares

En conclusión, la contribución relativa de los genes y el medio ambiente en el origen de la EoE ha sido analizada por varios estudios con diferentes enfoques, pero todos asignan un papel predominante a este último. Los factores de riesgo ambiental que conducen a la EoE y la forma en que interactúan con el huésped hacia la pérdida de la tolerancia inmunológica de la mucosa esofágica aún están por descubrirse. Sin embargo, este descubrimiento es imprescindible para proponer estrategias preventivas para al EoE. La función potencial y aún poco explorada de la microbiota esofágica en la mediación de la interacción entre el medio ambiente y el sistema de vigilancia de la mucosa esofágica aparece como uno de los enfoques más prometedores. Los cambios en la composición del microbioma esofágico en pacientes adultos y pediátricos con EoE en comparación con los controles también han sido descritos recientemente, a la vez que el efecto de los antibióticos sobre la microbiota durante las etapas primeras de la vida representa un factor de riesgo para el desarrollo de EoE.

Las muestras de biopsia de pacientes adultos con EoE activa muestran una carga bacteriana determinada por la expresión 16S aumentada, así como una sobreexpresión de varios TLRs en comparación con los controles, que revierten después del tratamiento dietético. Los mediadores de la inflamación de las vías de señalización de TLRs también están regulados al alza. Finalmente, las proteínas efectoras de la inmunidad innata también mostraron una mayor actividad. Todos estos cambios fueron corregidos tras inducir la remisión de la enfermedad mediante una intervención dietética.

Prevemos que la expansión epidemiológica de EoE y su amplio reconocimiento a través de múltiples poblaciones durante los próximos años facilitarán importantes avances en el conocimiento de los mecanismos íntimos de la enfermedad, en la optimización del diagnóstico y seguimiento de los pacientes y en el desarrollo de nuevos métodos que les permitan ser menos dependientes de la endoscopia. Además, podrán permitir un enfoque terapéutico hacia la medicina personalizada. Para ello, resulta indispensable seguir avanzando en desvelar la compleja fisiopatología de la EoE, definida por la interacción de múltiples células, moléculas, genes que interactúan con las exposiciones a factores ambientales durante las primeras etapas de la vida.

CONCLUSIONES

La EoE sigue aumentando en nuestro entorno, convirtiéndose en una enfermedad que afecta ya a más de un paciente por cada 1.000 habitantes, tanto entre niños como en adultos y cuya incidencia se iguala a la de la enfermedad inflamatoria intestinal.

El fenotipo predominante de los mastocitos esofágicos es MC_{TC} y su actividad expresada por la expresión de sus proteasas se relaciona con la actividad inflamatoria y con la severidad de los síntomas de la enfermedad.

Las vías de señalización dependientes de TLRs están activas en el esófago de los pacientes con EoE, apoyando la implicación de la microbiota y del sistema inmunitario innato en la patogénesis de la enfermedad, y revierten con el tratamiento dietético.

De manera específica, nuestros resultados permiten concluir que:

1 – La prevalencia de EoE en nuestro entorno asistencial localizado en una región central de España alcanzó en 2017 los 112 casos por 100.000 habitantes tanto en niños como en adultos, proporcionando las cifras globales más altas descritas hasta ahora. La incidencia media en niños para el periodo 2006 – 2017 fue de 10,6 nuevos casos por 100.000 habitantes/año, y de 9.1 nuevos casos por 100.000 habitantes/año en adultos. La mayor prevalencia se encuentra entre los 20 – 39 años y es más frecuente en varones que en mujeres. No encontramos variaciones estacionales en el diagnóstico de la EoE.

2 – La densidad de mastocitos y de eosinófilos en la mucosa esofágica es significativamente mayor en los pacientes con EoE activa que en los controles, y se asocia significativamente. Además, la densidad de ambos tipos celulares se correlacionó fuertemente con los síntomas de la enfermedad. El fenotipo MC_{TC} fue el predominante en la mucosa esofágica de los pacientes con EoE (representando en torno al 90% de los mastocitos), tanto en el epitelio como en la lámina propia del esófago.

3 – Todas las proteasas específicas de los mastocitos (triptasa, quimasa y carboxipeptidasa A3) muestran una expresión génica y proteica aumentada en los pacientes con EoE activa, respecto a los controles, y se normaliza tras el tratamiento dietético eficaz. Además, el nivel de expresión de estas proteasas también se correlacionó significativamente con la puntuación de síntomas de la enfermedad.

4 – Todas las eotaxinas implicadas en el reclutamiento de los eosinófilos activos (CCL11, CCL24 y CCL26) y su principal receptor (CCR3) se encontraron sobre-expresadas en los pacientes adultos con EoE. De manera paralela, la principal molécula quimiotáctica para los mastocitos (SCF), así como su receptor (SCFR) también estuvieron sobreexpresadas en las muestras de pacientes con EoE activa respecto a los controles.

5 – Los RNA mensajeros de TLR1, TLR2, TLR4 y TLR9 se encontraron sobreexpresados en la mucosa esofágica de los pacientes adultos con EoE en relación con los sujetos del grupo control. Paralelamente, TLR1, TLR2 y TLR4 se encontraron también sobreexpresados en las muestras del duodeno de los mismos pacientes. Los pacientes con EoE activa presentaron una carga bacteriana, determinada mediante RNA ribosómico 16S, aumentada en su esófago en comparación con la de los controles. No se observaron diferencias para las muestras de duodeno.

6 – Los principales adaptadores (MyD88, NF-κβ), mediadores (IL-1β, IL-6, IL-8 y IL-10) y efectores (PER-1, iNOS y GZMA) de la vía de señalización de TLRs se encontraron sobreexpresados en la mucosa esofágica de los pacientes adultos con EoE respecto a los controles. Estos cambios de expresión no se observaron en las muestras de duodeno.

7 – El tratamiento mediante dieta de eliminación empírica de 6 alimentos resultó efectivo para reducción hasta la normalidad la densidad de las células inflamatorias (eosinófilos y mastocitos), así como para revertir la expresión de las citoquinas y proteasas específicas de mastocitos y de las moléculas implicadas en los mecanismos de transducción de señales dependientes de TLRs.

8 – Hemos propuesto mediante este trabajo una hipótesis fisiopatológica para la EoE, que parte de la disregulación de la tolerancia inmunológica determinada por cambios en el microbioma, destaca la función inmunoreguladora del epitelio y la pérdida de la función de la barrera mucosa como principal factor para el mantenimiento de la respuesta inflamatoria. Los componentes del sistema inmunitario innato participan en el inicio, en la cronificación y en los fenómenos de remodelación del esófago en la EoE.

RESUMEN

Este trabajo tiene como objetivo conocer la epidemiología de la esofagitis eosinofílica (EoE) y su evolución a lo largo de los últimos años, caracterizar el infiltrado inflamatorio esofágico por mastocitos, en cuanto a su fenotipo y asociación con las manifestaciones clínicas de la EoE y, por último, también pretende definir la respuesta innata esofágica mediada por *toll-like receptors* (TLR) en la enfermedad y su regulación mediante el tratamiento dietético.

Para conocer la epidemiología de la EoE se desarrolló un estudio de base poblacional en un área sanitaria de Castilla-La-Mancha, donde se registraron prospectivamente todos los pacientes diagnosticados de EoE durante los últimos 12 años (2006 – 2017). Se documentó un aumento rápido en la frecuencia de la EoE. La prevalencia de la EoE fue de 111,9 casos por 100.000 habitantes (tanto en niños como en adultos), por lo que la EoE estaría presente en uno de cada 893 habitantes. La incidencia media anual fue de 10,6 y 9,1 nuevos casos/100.000 habitantes y año para niños y adultos, respectivamente. La mayoría de los pacientes diagnosticados de EoE lo fueron entre los 20 y 24 años y entre los 35 y 39 años, con predominio entre los varones y sin una tendencia estacional en el momento de su diagnóstico.

La caracterización del infiltrado por mastocitos y de la respuesta innata mediada por TLRs se llevó a cabo mediante un estudio cuasi-experimental en pacientes con EoE, analizados antes y después de un tratamiento dietético empírico consistente en una eliminación de los seis principales grupos de alimentos causantes de la enfermedad. Los resultados fueron comparados con los de un grupo control.

Nuestros resultados muestran que los mastocitos mantienen una estrecha interacción con los eosinófilos, siendo elementos fundamentales en el infiltrado inflamatorio de la EoE. Observamos un aumento en la densidad de mastocitos, la expresión de sus receptores y los niveles de expresión de sus principales proteasas (triptasa, quimasa, carboxipeptidasa A3). Éstos a su vez correlacionaron con la intensidad de los síntomas. Los mastocitos MC_{TC} fueron el fenotipo predominante en la EoE, representando más del 90% de estas células.

Pudimos también documentar la activación del sistema inmune innato en los pacientes con EoE activa, quienes poseen también una mayor carga bacteriana, una alteración en la expresión de mucinas (inhibición de la expresión de Muc1 y Muc5B), y que se caracteriza por una sobre-expresión de los TLRs (TLR1, TLR2, TLR4 y TLR9, tanto génica como proteica de entre 3 y 4 veces sobre el nivel de los controles). Los factores de transcripción implicados en sus vías de señalización (MyD88 y NF- κB, que duplicaron su expresión), las citoquinas (IL-1β, IL-6, IL-8 e IL-10) y efectores (PRF-1, iNOS y GZMA) de la inflamación también se observaron sobre-expresados. Estos últimos fenómenos se localizaron exclusivamente en el epitelio esófagico, siendo excluidos en la mucosa duodenal no inflamada.

El tratamiento dietético redujo el nivel expresión génica de las moléculas analizadas y la densidad de mastocitos y eosinófilos en los pacientes con EoE a niveles similares a los controles.

ABSTRACT

The aim of this study was to calculate the epidemiology of eosinophilic esophagitis (EoE) and its evolving trends over the last years, to characterize the esophageal inflammatory infiltrate by mast cells, in terms of its phenotype and association with clinical manifestations of the disease. Finally, we aimed to define toll-like receptors (TLR)-mediated innate esophageal responses involved in the disease and regulation through dietary treatment.

To estimate the epidemiology of EoE, a population-based study was conducted in a health area of Castile-La Mancha, were all patients suffering the disease were prospectively enrolled during the last 12 years (2006 - 2017). A sharp increase in the frequency of EoE was documented. The prevalence of EoE was 111.9 cases per 100,000 inhabitants (both in children and adults). Therefore, EoE would affect to one out of 893 inhabitants. Average annual incidence was 10.6 and 9.1 new cases / 100,000 inhabitants and year for children and adults, respectively. The highest prevalences were observed in ages ranging between 20 and 24 and 35–39 years old, with predominance in male. No seasonal trend in the moment of diagnosis was found.

The characterization of the mast cell infiltrate and the innate response mediated by TLRs was carried out by a quasi-experimental study in patients with EoE, assessed before and after dietary therapy with empiric elimination of the six main food groups that cause the disease. Results were compared with those obtained from a control group.

Our results show that mast cells maintain a close interaction with eosinophils, being basic elements within the inflammatory infiltrate of EoE. An increase in mast cell density, upregulation of expression in its receptors and increased expression levels of main proteases (tryptase, chymase, carboxypeptidase A3) were observed. Additionally, all of them correlated with symptom scores. The MCTC phenotype was the predominant one in EoE representing more than 90% of mast cells.

We also documented activation of the innate immune system in patients with active EoE, who presented a higher bacterial load, an altered expression of mucins (with downregulation of Muc1 and Muc5B), and upregulation of TLRs (TLR1, TLR2, TLR4 and TLR9, both at gene and protein expression, between 3 and 4 folds over the controls). Transcriptional factors involved in TLR-mediated signaling pathways (MyD88 and NF-κB that duplicate their expression), cytokines (IL-1β, IL-6, IL-8 and IL-10) and effector molecules (PRF-1, iNOS and GZMA) were also upregulated. These last phenomena located exclusively in the esophageal epithelium, being excluded in the non-inflamed duodenal mucosa.

Dietary treatment downregulated gene expression levels of all molecules in esophageal biopsies of patients with EoE, as well as the density of mast cells and eosinophils to levels similar to control subjects.

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ANEXOS

Anexo I: Relación de figuras incluidas en el texto

- Figura 1: Clasificación y graduación de los hallazgos endoscópicos en la esofagitis eosinofílica.

- Figura 2: Dispositivos mínimamente invasivos utilizados en EoE. **A** Citoesponga. **B** String Test.

- Figura 3: Algoritmo terapéutico para el manejo de la EoE

- **Figura 4:** Modelo genético explicativo integrado de la EoE

- Figura 5: Inter-relación entre el sistema inmune innato y adaptativo

- **Figura 6:** Tipos y localización de los TLRs

- Figura 7: Vías de transducción de señales de los TLRs.

- Figura 8: Nueva hipótesis integrativa de la fisiopatología de la EoE y sus mecanismos celulares y moleculares

Anexo II: Relación de tablas incluidas en el texto.

- Tabla 1: Principales estudios epidemiológicos de base poblacional realizados en EoE.

- Tabla 2: Principales estudios realizados con dietas elementales para el tratamiento de EoE.

- Tabla 3: Principales estudios realizados con dietas dirigidas por pruebas de alergia para el tratamiento de EoE.

- Tabla 4: Principales estudios realizados con dietas empíricas de eliminación para la EoE.

- Tabla 5: Principales estudios realizados con IBPs para el tratamiento de pacientes con EoE.

- Tabla 6: Principales ECA realizados con esteroides tópicos para el tratamiento de pacientes con EoE.

- Tabla 7: Principales estudios realizados con dilataciones para el tratamiento de pacientes con EoE con medición de mejoría clínica.

- Tabla 8: TLRs descritos en humanos, su ubicación celular y ligandos conocidos.

Anexo III: Artículos publicados por el doctorando relacionados con el tema de investigación

- Lucendo AJ, Arias-González L, Molina-Infante J, **Arias Á**. ***Determinant factors of quality of life in adult patients with eosinophilic esophagitis.*** United European Gastroenterol J. 2018 Feb;6(1):38-45. doi: 10.1177/2050640617707095. Epub 2017 Apr 21.

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Anexo IV: Documento del Comité de ética para la Investigación Clínica (CEIC)

Complejo Hospitalario La Mancha Centro

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INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Dª Esperanza Segura Molina, Secretaria del Comité Ético de Investigación Clínica del Complejo Hospitalario La Mancha Centro

CERTIFICA

Que este Comité ha evaluado la propuesta para la investigación clínica del estudio titulado: ***"Caracterización y estudio de los mastocitos en el infiltrado inflamatorio de la esofagitis eosinofílica: Definición de la función fisiopatológica de una posible diana terapéutica",*** cuyo Investigador Principal es el Dr. Alfredo José Lucendo Villarín, del Servicio de Digestivo, en su reunión del día 23/10/08; Acta 10/2008 y en cuya valoración estuvieron presentes los siguientes miembros del C.E.I.C.:

Secretaria: Dra. Esperanza Segura Molina (Farmacóloga Clínica)
Dr. Alfonso Gimeno Collado (Urología)
D. Ignacio Alcañiz Octavio (Enfermero)
Dra. Dolores Fraga Fuentes (Farmacia)
D. Alipio Lara Olivares (Director Instituto Vid Vino de Castilla La Mancha)
Dr. Laureano Gómez González (Atención Primaria)
D. José Mª Tenías Burillo (Unidad de Apoyo a la Investigación)

EVALUACIÓN: FAVORABLE

Lo que firmo en Alcázar de San Juan, a 27 deOctubre de 2.008

LA MANCHA CENTRO - COMPLEJO HOSPITALARIO - COMITE ETICO INVESTIGACIÓN CLINICA

Fdo: Dra. Esperanza Segura Molina
Secretaria C.E.I.C.

Anexo V: Documento del Comité de ética para la Investigación Clínica (CEIC)

Complejo Hospitalario La Mancha Centro

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INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Dª Esperanza Segura Molina, Secretaria del Comité Ético de Investigación Clínica del Complejo Hospitalario La Mancha Centro

CERTIFICA

Que este Comité ha evaluado la propuesta para la realización del estudio de investigación clínica titulado: ***"Caracterización y expresión de TLRs (TOLL LIKE RECEPTORS) en la esofagitis eosinofílica: Estudio de un nuevo mecanismo fisiopatológico"*** cuyo investigador principal es el Dr. Alfredo José Lucendo Villarín, del Servicio de Digestivo del Hospital de Tomelloso, en su reunión del pasado 22 de julio de 2010; Acta núm. 07/10 y en cuya valoración estuvieron presentes los siguientes miembros del C.E.I.C.:

Presidente: Dr. Francisco Pérez Roldán (Digestivo)
Secretaria: Dra. Esperanza Segura Molina (Farmacóloga Clínica)

Vocales:
Dra. Dolores Fraga Fuentes (Farmacia)
Dr. José Mª Tenías Burillo (Unidad de Apoyo a la Investigación)
Dra. Concepción Villafáñez García (Urgencias)
Dr. Ramón Garrido Palomo (Pediatría)
D. Alipio Lara Olivares (Instituto de la Vid y el vino)
Dª Ana Mª Jiménez Ruiz (Suministros-Logística)
D. Ángel Arinero Arinero (Asesoría Jurídica)

EVALUACIÓN: FAVORABLE

Lo que firmo en Alcázar de San Juan, a 23 de julio de 2010

COMITÉ ÉTICO INVESTIGACIÓN CLÍNICA

Fdo: Dra. Esperanza Segura Molina
Secretaria C.E.I.C.